GENETIC RESOURCES, CHROMOSOME ENGINEERING, AND CROP IMPROVEMENT SERIES

Volume 1

GENETIC RESOURCES, CHROMOSOME ENGINEERING, AND CROP IMPROVEMENT

Grain Legumes

EDITED BY
RAM J. SINGH AND PREM P. JAUHAR

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Dedication

Johann Gregor Mendel 1822–1884

This book is dedicated to the memory of Johann Gregor Mendel, whose simple but elegant experiments on hybridization with garden pea (Pisum sativum *L.*) *led to the foundation of the science of genetics, which has played a vital role in agriculture, biology, and medicine. He was an unsung hero whose pioneering work of extraordinary importance was not recognized in his own lifetime.*

Preface

Cereal crops—mainly wheat, rice, maize, pearl millet, and sorghum—constitute the primary food source of the world population. While cereals are an excellent source of energy in the form of carbohydrates, grain legumes (common bean, pea, pigeonpea, cowpea, lentil, chickpea, and others) (family Fabaceae) are the major, if not the only, source of dietary protein for a large proportion of the population in Asia, Africa, and other impoverished regions. Seeds of primary grain legumes are consumed as *daal* and soup, while flour is used for preparing snacks or mixed with cereal flour for baking wholesome "chapattis." Legumes also play a positive role in crop rotations with cereals and help replenish soil's nitrogen supply. Their characteristic root nodules harbor the rhizobial bacteria that bring about nitrogen fixation in the soil. Cereals and legumes form a unique combination in the diet of the poor, who cannot afford animal protein. Thus, grain legumes are sometimes called the poor man's meat. They are the primary source of protein for people (predominantly in India) who are strictly vegetarian and will not consume any form of animal protein. In addition to their rich protein content, pulses such as chickpea have a unique combination of nutrients, including iron, calcium, and zinc, as well as members of the vitamin B complex, which makes them an ideal component of the human diet. According to Nancy Longnecker at the Centre for Legumes in Mediterranean Agriculture at The University of Western Australia (http://www.research.deakin.edu.au/), "Eating more legumes lowers the risk of heart disease, type-II diabetes, and obesity." She further stated that a U.S. study lasting 19 years involving 9600 men and women showed that those who "ate four or more servings of legumes (including chickpea) per week were 22% less likely to suffer from coronary heat disease than those who ate less than one serving per week." Therefore, researchers around the globe are constantly in search of legumes as a supplement in improving modern human diets. Despite their nutritional superiority and great importance to human health, grain legumes have not received the attention they deserve—in fact, not even half the attention cereals have claimed. It is ironic that although pea (*Pisum sativum* L.) formed the experimental material in the seminal hybridization work of the founder of genetics, Johann Gregor Mendel, in the 1860s, genetic improvement of this important legume has lagged far behind that of other crops. The importance of bringing about genetic improvement of leguminous crops cannot be overemphasized.

Improving yields of the grain legumes remains a primary breeding goal of various national and international programs. Many of these centers maintain germplasm resources as potential donors of genes for resistance to various biotic and abiotic stresses. Because there is no consolidated account of germplasm resources and cytogenetic manipulation and breeding of grain legumes, we planned to bring out such a book that constitutes volume I in a series on "germplasm resources, chromosome engineering, and

crop improvement." The idea of bringing out this series of five volumes, each dealing with grain legumes, cereals, oilseed crops, vegetable crops, and forage crops, was first conceived by one of us (Singh), and world-renowned scientists were invited to contribute chapters on various crops. This volume consists of 11 chapters dealing with major grain legumes of great economic importance to developing countries and to the developed world. These chapters give comprehensive and authoritative accounts of genetic resources and their utilization for improving yields, disease, and pest resistance—and other agronomic traits of the most widely grown and consumed legumes.

The introductory chapter summarizes the landmark research done in ten leguminous crops, giving, in tabulated form, information on germplasm availability for breeding for high yields and improved protein content. Appropriate germplasm collections can be a good source for genetic enhancement of various traits in grain legumes and for broadening their genetic base. Each of the subsequent chapters, 2–11, deal, respectively, with one of the 10 crops: common bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.), pigeonpea [*Cajanus cajan* (L.) Millsp.], cowpea [*Vigna unguiculata* (L.) Walp.], faba bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), lupin (*Lupinus* L.), mungbean [*Vigna radiata* (L.) Wilczek], and azuki bean [*Vigna angularis* (L.) Ohwi & Ohashi]. Each chapter provides comprehensive information on the origin of the crop, its genetic resources in various gene pools, basic cytogenetics, conventional breeding, and the modern tools of molecular genetics and biotechnology. The primary (GP-1), secondary (GP-2), and tertiary (GP-3) gene pools of each crop are identified. Utilization of these resources in producing high-yielding cultivars with resistance to biotic and abiotic stresses is also described. In view of the narrow genetic base of various legumes, several authors have recommended the use of GP-2 and GP-3 resources in producing widely adapted varieties by wide hybridization. In addition to superior nutritional components stated above, some grain legumes contain antinutritional elements, and the authors have recognized such undesirable traits in the crops they have dealt with. There is ongoing research to produce varieties without—or with only low amounts of—antinutritional elements by conventional breeding and transgenic technology.

Each chapter has been written by one or more experts in the field. We are extremely grateful to all the authors for their invaluable contributions. We have been fortunate to know them both professionally and personally. We are also very grateful to scientists who reviewed various chapters. Our communications were always cordial and friendly. We are particularly indebted to Daniel Debouck, Ian Dundas, James Kelly, Tanveer Khan, Phil Miklas, Srinivas Rao, Bob Redden, Fred Muehlbauer, José Cubero, Kadambot Siddique, Govindjee, and Deoki Tripathy for their comments and suggestions on some of the chapters. Although every chapter has been appropriately reviewed by experts in the field, the authors are ultimately responsible for the accuracy and completeness of their respective chapters. One of us (Singh) would like to thank Dr. Steven G.Pueppke, Associate Dean and Research Director at the University of Illinois, Urbana, for all his support and encouragement.

This book is intended for professionals and graduate students whose interests center upon genetic improvement of crops in general, and grain legumes in particular. The book will benefit plant breeders, agronomists, cytogeneticists, taxonomists, molecular biologists, and biotechnologists. Graduate-level students in these disciplines with

adequate background in genetics and a spectrum of other researchers interested in biology and agriculture will also find this volume a worthwhile reference. We sincerely hope that the information in this book will help in the much-needed genetic amelioration of grain legumes.

Ram J.Singh
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The Editors

Ram Jag Singh, M.Sc., Ph.D., is an agronomist-plant cytogeneticist in the Department of Crop Sciences, University of Illinois at Urbana-Champaign. He received his Ph.D. in plant cytogenetics under the guidance of the late Professor Takumi Tsuchiya from Colorado State University, Fort Collins. He benefited greatly from Dr. Tsuchiya's expertise in cytogenetics.

Dr. Singh conceived, planned, and conducted pioneering research related to cytogenetic problems in barley, rice, rye, wheat, and soybean. Thus, he isolated monotelotrisomics and acrotrisomics in barley, identified them by Giemsa C- and N-banding techniques and determined chromosome arm-linkage group relationships. In soybean *(Glycine max)*, he produced fertile plants with 2n=40, 41 or 42 chromosomes, from an intersubgeneric cross between soybean and a wild species, *G. tomentella* (2n=78), and obtained certain lines with resistance to the soybean cyst nematode (SCN). Dr. Singh constructed, for the first time, a soybean chromosome map based on pachytene chromosome analysis, which laid the foundation for creating a global soybean map. By using fluorescent genomic *in situ* hybridization, he confirmed the tetraploid origin of the soybean.

Dr. Singh has published 65 research papers, mostly in reputable international journals, including *American Journal of Botany, Chromosoma, Crop Science, Genetics, Genome, Journal of Heredity, Plant Breeding,* and *Theoretical and Applied Genetics.* In addition, he summarized his research results by writing several book chapters. He has contributed nine book chapters, and has presented research findings as speaker at national and international meetings. His book on plant cytogenetics is widely used for teaching graduate students. He is a member of the Crop Science Society of America and the American Society of Agronomy. In 2000, Dr. Singh received the Academic Professional Award for Excellence: Innovative & Creativity from the University of Illinois at Urbana-Champaign.

Prem Prakash Jauhar, M.Sc., Ph.D., is a senior research geneticist with the U.S.Department of Agriculture—Agricultural Research Service, Northern Crop Science Laboratory in Fargo, North Dakota. He also holds the position of adjunct professor of cytogenetics with North Dakota State University, Fargo. He is the principal investigator on the USDA project Genomic Relationships in the Triticeae and Enhancement of Wheat Germplasm by Classical and Molecular Techniques.

Dr. Jauhar earned his Ph.D. from the Indian Agricultural Research Institute, New Delhi, in 1963, when he was appointed to the faculty, and worked there until 1972 doing research and teaching cytogenetics to graduate students. He then served as a senior

scientific officer at the University College of Wales, Welsh Plant Breeding Station, Aberystwyth, Wales. In 1976, he immigrated to the United States.

Dr. Jauhar's research interests have centered on various facets of cytogenetics and biotechnology and their relevance to plant breeding. He has been particularly interested in chromosome pairing. He discovered the regulatory mechanism that controls chromosome pairing in polyploid species of *Festuca* [*Nature* (London) 254, 595–597, 1975] and originated the concept of hemizygous-ineffective genetic control of pairing—a phenomenon that has major implications in cytogenetics, plant breeding, and evolution. His other research interests include work on aneuploids, polyploids, haploids, B chromosomes, induced mutagenesis, wide hybrids, and genome analysis. After establishing an efficient *in vitro* regeneration system for durum wheat, his lab produced the first transgenic durum wheat and standardized the technology of direct gene transfer into scutellar cells (*Journal of Heredity* 88, 475–481, 1997). This transgenic technology paved the way for direct gene transfer into commercial durum cultivars and opened up new avenues of germplasm enhancement. He is also involved in germplasm enhancement by genomic reconstitution through wide hybridization coupled with manipulation of homoeologous chromosome pairing. By transferring part of a wild grass chromatin into the durum wheat genome, Dr. Jauhar produced durum germplasm with scab resistance (*Euphytica* 118, 127–136, 2001). This technique offers an excellent option for the production of scabresistant durum cultivars.

Working on *ph1*-and *ph1b*-euhaploids in bread wheat (2n=3x=21; ABD genomes) and durum wheat (2n=2x=14; AB genomes) that he synthesized, Dr. Jauhar elucidated inter- and intragenomic relationships in these polyploid wheats. He demonstrated that the A and D genomes of bread wheat are more closely related to each other than either one is to B—a finding that contributed to the understanding of the phylogeny of wheat. His haploidy research produced the first clear evidence of sexual polyploidization via 2n gamete formation in durum wheat haploids (*Crop Science* 40, 1742–1749, 2000), demonstrating how polyploids are produced in nature. By producing substitution haploids of durum, his team elucidated a part of the evolutionary cyclic translocation 4A·7B, which occurred some 500,000 years ago at the time of origin of tetraploid emmer wheat (*Genome* 44, 137–142, 2001). The method of producing durum haploids by wide hybridization standardized in his lab has been incorporated in the first *Manual on Haploid and Double Haploid Production in Crop Plants* (Kluwer Academic Publishers, The Netherlands (2003).

Dr. Jauhar has published in prestigious international journals, including *Nature, Chromosoma, Theoretical and Applied Genetics, Genome, the Journal of Heredity, Genetica, Plant Breeding, Mutation Research, Hereditas,* and *Molecular and Environmental Mutagenesis*. He has 120 publications, including 90 research papers, three books (two authored, and one co-authored and edited) by prestigious publishers, and 17 book chapters. His research papers and books are used in graduate teaching and research worldwide. He has given seminars in several countries, organized and chaired symposia and scientific sessions at national and international conferences, and served on international advisory committees. He served on the International Advisory Committee for the 13th International Chromosome Conference held in Italy, September 8–12, 1998. Since 1991, he has served as an associate editor of the *Journal*

of Heredity. He also referees numerous research manuscripts submitted to other international journals.

Dr. Jauhar has received several awards and professional recognitions. Some recent awards include his election as Fellow of three major societies: the Crop Science Society of America (1995), the American Society of Agronomy (1996), and the American Association for the Advancement of Science (2002).

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CHAPTER 1
Landmark Research in Grain Legumes

Ram J.Singh

1.1 INTRODUCTION

The primary dietary grain legumes included in this series are the common bean (*Phaseolus vulgaris* L.), cowpea [*Vigna unguiculata* (L.) Walp.], pigeonpea [*Cajanus cajan* (L.) Millsp.], chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), lentil (*Lens culinaris* Medik.), mungbean [*Vigna radiata* (L.) Wilczek], azuki bean [*Vigna angularis* (L.) Ohwi & Ohashi], and pea (*Pisum sativum* L.). Several species of lupin are used primarily for animal feed in Australia and as a forage crop in Europe. The hallmark trait of legume species is their high protein content (see Table 1.1).

Grain legumes and cereals co-evolved in a symbiotic way. They are complementary components of agricultural systems worldwide, including common bean and maize in South America; lentil, pea, chickpea, faba bean, bitter vetch with two wheats (durum and einkorn), as well as barley in the Middle East; soybean with millet in North China; and *Vigna* bean (and soybean-rice, later) in

South China (José Cubero; personal communication, May 26, 2004). In Africa, cowpea grows with pearl millet and sorghum.

Grain legumes are members of the family Fabaceae. The major agricultural legumes are divided into two main groups. The warm-weather group contains *Vigna*, *Phaseolus*, *Cajanus*, and *Glycine*. The cool-season group includes *Vicia*, *Pisum*, *Trifolium*, and *Lotus* (see Chapter 11). This volume includes major grain legumes of both groups that are used for food and feed. The terms *grain legume* and *pulse* need clarification. *Grain legume* refers to the legume species of which the edible part is seed (food and feed). Pulse is derived from the Latin word that means “pottage” and mainly refers to food legumes. Soybean [*Glycine max* (L.) Merr.] and groundnut (peanut) (*Arachis hypogea* L.) were, in fact, pulses at the very beginning. However, now they are considered oilseed crops because they contain more than 20% oil and are used extensively for oil and meal (K. Siddique; personal communication, May 18, 2004).

The characteristic feature of legumes is the presence of root nodules, which contains the bacterium *Rhizobium*, and related genera, that helps nitrogen fixation in the soils, maintaining a symbiotic relationship. However, such bacterial association is absent in cereals. Grain legumes are rich in protein (20 to 50%), while cereals are an excellent source of carbohydrates. The combination of cereals and grain legumes enriches the

human diet, especially when supplementing the protein requirement. Grain legumes are an important source of protein in countries where the majority of people are vegetarian both by choice and due to religious beliefs, such as India. However, in Central America and the Caribbean, rice and beans is a staple dish, even though most people are not vegetarian.

Grain legumes are second only to cereals in their dietary importance to humans and animals (Graham and Vance, 2003). Although grain legumes are an extremely valuable source of protein for both humans and animals, research efforts for producing high-yielding cultivars of grain legumes lag far behind that of cereals. The poor yield of grain legumes may be due to the growing of inherently unproductive cultivars that are not tolerant to abiotic and biotic stresses. Grain legumes are often cultivated as subsistence crops in smallholdings and for home consumption as part of the "kitchen garden." Compared with cereals, research on pulse crops has been largely neglected in developing countries. This chapter summarizes landmark research efforts in 10 major grain legumes.

1.2 ESTABLISHMENT OF INTERNATIONAL AND NATIONAL PROGRAMS

The following international and national centers have been established for major grain legume research:

1. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia (http://www.ciat.cgiar.org/): Common bean is a mandate crop.
2. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India (http://www.icrisat.org/): Pigeonpea and chickpea are mandate crops.
3. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (http://www.iita.org/): Cowpea is a mandate crop.
4. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria (http://www.icarda.org/): Chickpea, lentil, and faba bean are mandate crops. The center also maintains pea germplasm but has no active varietal improvement programs.
5. Asian Vegetable Research and Development Center (AVRDC), Taiwan (http://www.avrdc.org/): Mungbean is a mandate crop, and the center has the largest germplasm collection of this crop. Major collections of the related azuki bean are held at AVRDC and in national collections of China and Japan.
6. National Programs: National (public) and private industries worldwide have legume improvement programs.

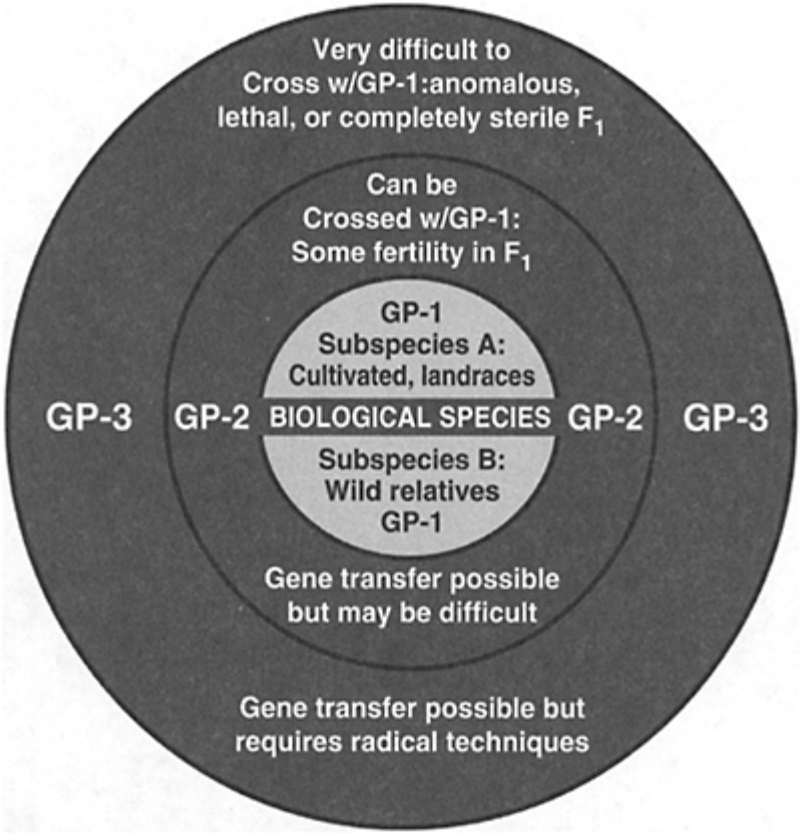

Figure 1.1 Gene pool concept in plants established based on hybridization. (Modified and redrawn from Harlan and de Wet, 1971.)

1.3 GENE POOLS FOR GRAIN LEGUMES

The gene pool concept developed by Harlan and de Wet (1971) has played a pivotal role in the utilization of germplasm resources for producing high-yielding cultivars without antinutritional chemicals by conventional methods and by transformation technology. Harlan and de Wet (1971) proposed three gene pool concepts based on the results of hybridization among species. These are primary (GP-1), secondary (GP-2), and tertiary (GP-3) (Figure 1.1).

1.3.1 Primary Gene Pool

The primary gene pool (GP-1) of grain legumes consists of landraces and biological species. Crossing within this gene pool is easy and hybrids are vigorous, exhibit normal meiotic chromosome pairing, and possess total fertility. The gene segregation in F_1 is normal and gene exchange is generally easy. Primary gene pool A includes cultivated races and landraces. Primary gene pool B includes subspecies, wild and weedy relatives (see Figure 1.1). Table 1.1 lists the primary gene pool of grain legumes.

1.3.2 Secondary Gene Pool

The secondary gene pool (GP-2) includes all species that can be crossed with GP-1 with at least some fertility in F_1s (see Figure 1.1). Gene transfer is possible with some difficulty. In this

Table 1.1 **Common Name, Scientific Name, 2n Chromosome Number, Origin, and Gene Pools of Major Grain Legumes**

Common Name	Scientific Name	2n	Origin	Gene Pool			% Protein Content
				GP-1	GP-2	GP-3	
Common bean	*Phaseolus vulgaris*	22	Mexico, Middle American, and Andean South America	Domesticated cultigens, wild populations	*P. coccineus*	*P. acutifolius*	22; Chapter 2; Singh,
					P. polyanthus	*P. parvifolius*	S.P.
					P. costaricensis		
Pea	*Pisum sativum*	14	Fertile Crescent, the Mediterranean, and Central Asia	Domestic cultigens wild relative subspecies	*P. fulvum*	None	26; Chapter 3; Redden et al.
				P. pumilio			
				P. elatius			
				P. abyssinicum			
Pigeonpea	*Cajanus*	22	India	Cultivated	*C. acutifolius*	*C. cinereus*	20–22;

	cajan			landraces			Chapter 4; Saxena, K.B.
					C. albicans	*C. confertiflorus*	
					C. cajanifolius	*C. crassus*	
					C. confertifolius	*C. geonsis*	
					C. lanceolatus	*C. latisepalus*	
					C. lineatus	*C. mollis*	
					C. reticulatum	*C. platycarpus*	
					C. scarsbseoides	*C. rugosus*	
					C. sericeus	*Dunbaria* spp.	
					C. trinervius	*Rhynchosia* spp.	
Cowpea	*Vigna unguiculata*	22	Africa	Four cultigroups, landraces, and subsp. *tenuis denkindtiana, stenophylla*	Subsp. *pubescence*	*V. vexillata*	20–26; Chapter 5; Singh, B.B.
						V. radiata	
Faba bean	*Vicia faba*	12	Near East	Domestic cultigens	None	None	25–33; Chapter 6; Cubero and Nadal
Chickpea	*Cicer arietinum*	16	Southern Caucasus, northern Persia, and southeastern Turkey	*C. arietinum*	*C. bijugum*	*C. chorassanicum*	23; Chapter 7; Ahmad et al.
				C. echinospermum	*C. judaicum*	*C. cuneatum*	
				C. reticulatum	*C. pinnatifidum*	*C. yamashitae*	
						All perennial 34 *Cicer*	

						species	
Lentil	*Lens culinaris*	14	Near East arc and Asia Minor	subsp. *culinaris*	*L. ervoides*	*L lamottei*	26; Chapter 8; Muehlbauer, F.J.
				subsp. *odemensis*	*L. nigricans*	*L. tomentosus*	
				subsp. *orientalis*			
Lupin	*Lupinus albus*	50	Mediterranean	*L. albus* var. *albus*	*L. micranthus*	All other Old and New	36.1; Chapter 9; Clements et al.
				L. albus var. *graecus* (wild form)	? Western New World Lupinus	World *Lupinus*	
	Lupinus luteus	52	Mediterranean	Wild forms, landraces, cultivars ssp. *orientalis*	L. *hispanicus* ssp. bicolor ssp. *hispanicus*	All other Old and New World *Lupinus*	38.3; Chapter 9; Clements et al.
	Lupinus angustifolius	40	Mediterranean	All wild, land race, and domesticated forms of *L. angustifolius*	*L. luteus*	All other Old and New World *Lupinus*	32.2; Chapter 9, Clements et al.
					L. *hispanicus*		
	Lupinus cosentinii	32	Mediterranean	*L. digitatus*	*L. palaestinus*	All other Old World and New World *Lupinus*	32.1; Chapter 9; Clements et al.
				L. atlanticus	*L. princei*		
				L. pilosus	*L. somaliensis*		
	Lupinus mutabilis	48	South America	?Western, North, and South American species with 2n=48	*L. albus*	All other Old World and Western New *World Lupinus*	44.0; Chapter 9; Clements et al.
					L. micranthus		
Mungbean	*Vigna*	22	India	*V. radiata*	*V. mungo*	Section	22.9;

	radiata			var. *radiata*		*Aconitifoliae*	Chapter 10; Tomooka et al.
				V. radiata var. *sublobata*	*V. subramaniana*	Section *Angulares*	
						V. stipulacea	
						V. grandiflora	
Azuki bean	*Vigna angularis*	22	Asia	*V. angularis* var. *angularis*	*V. umbellata*	Section *Ceratotropis*	21.1; Chapter 11; Vaughan et al.
						V. trinervia	
				V. angularis var. *nipponensis*			
				V. hirtella			
				V. minima			
				V. nakashimae			
				V. nepalensis			
				V. riukiuensis			
				V. tenuicaulis			

regard, GP-2 for common bean, pigeonpea, chickpea, lentil, mungbean, and azuki bean is availableand can be used in varietal improvement. Cowpea and faba bean do not have a GP-2, and thereare no GP-1 relatives of faba bean (see Table 1.1).

1.3.3 Tertiary Gene Pool

The tertiary gene pool (GP-3) is the extreme outer limit of potential genetic resource (see Figure 1.1). Table 1.1 lists GP-3 of pigeonpea, cowpea, chickpea, lentil, lupin, mungbean, and azuki bean. Pea and faba bean are without GP-3 (see Table 1.1). Prezygotic and postzygotic barriers can cause partial or complete failure of hybridization,

inhibiting introgression between GP-1 and GP-3. The exploitation of wild relatives of grain legumes is often hampered by poor crossability, early embryo abortion, hybrid inviability, hybrid seedling lethality, and hybrid sterility due to low chromosome pairing. Technology to exploit GP-3 for broadening the genetic base of grain legumes is yet to be developed.

1.4 GERMPLASM RESOURCES FOR GRAIN LEGUMES

The international and national institutes for grain legumes (common bean, pea, pigeonpea, cowpea, faba bean, chickpea, lentil, lupin, mungbean, azuki bean) collect, maintain, disseminate, and develop breeding lines with resistance to abiotic and biotic stresses. Plant exploration of wild relatives, often described as "exotic" germplasm, of common bean, faba bean, lentil, chickpea, and cowpea is extensive. Although pea is an important legume, it does not have an international institute for its research, but several national research institutes maintain active breeding programs (see Chapter 3). The National Institute of Agrobiological Sciences in Japan has a very active research program for the Asian *Vigna*, which includes mungbean (see Chapter 10) and azuki bean (see Chapter 11).

Wild relatives of major grain legumes included in this volume are being characterized based on classical taxonomy, cytogenetics, and molecular methods. The combination of the genus *Atylosia* with the genus *Cajanus* is a classic example (van der Maesen, 1986; Chapter 4).

Cytogenetics of grain legumes has not progressed as rapidly as for cereals, although the foundation of genetics was laid by Johann Gregor Mendel's pea experiments. Simultaneously, Galton developed "Biochemical or Quantitative Genetics" by using *Lathyrus odoratus*, which was then a garden plant. Cytogenetics of major grain legumes is lacking—the only exception being faba bean. Taylor et al. (1957) demonstrated semiconservative replication of *Vicia faba* chromosomes by using tritium-labeled thymidine. Faba bean and onion root tips were used for studying cell division and cytogenetics because their chromosomes are large, few in number, and stain very well. They were the model crops used to study the effect of chemicals on chromosome structure.

Cytogenetic stocks and molecular maps of grain legumes are being developed in common bean and faba bean. A composite molecular map has been successfully developed including morphological markers, isozymes, random amplified polymorphic DNAs (RAPDs), sequence-characterized amplified regions (SCARs), seed protein genes, and microsatellites. Using trisomics, the linkage groups of faba bean have been placed in their respective chromosomes; for the long metacentric chromosome, whose trisomics could not be obtained, some markers were developed to build up its linkage map. The linkage groups have so far been obtained to cover about 1600cM with an overall map interval of 8cM. Several important characters have been mapped, such as genes and quantitative trait loci (QTLs) for resistance to ascochyta, rust, and broomrape resistance, as well as for the two main antinutritional factors. In this map, genes controlling important characters of both qualitative (Mendelian) and quantitative (QTLs) natures are

being placed. Marker-assisted selection (MAS) and studies on synteny are breeders' ultimate objectives (see Chapter 6).

The impact of somaclonal variation and genetic transformation for producing better grain legumes is limited. Genetically modified grain legumes are being produced in several laboratories by transformation; however, they have not been released for commercial production.

Some high-yielding legume cultivars are eroding the natural habitat of the allied species and genera. It is very important, therefore, that these invaluable germplasm resources are collected before they become extinct. The international and national institutes are preserving indigenous varieties, landraces, and wild relatives in medium- and long-term storages and their viability is checked routinely.

1.5 GERMPLASM ENHANCEMENT FOR GRAIN LEGUMES

The genetic base of grain legumes is rather narrow because breeders have been confined in their crop improvement programs to GP-1 (primitive cultivated forms, landraces, and wild progenitors). Although GP-2 has been used to improve common bean, it is beyond reach for improving lentil (see Chapter 8). GP-3 has not been exploited to introgress traits of economic importance in cultivated legume species. A large number of exotic accessions are stored in seed banks worldwide (Tanksley and McCouch, 1997). However, only a fraction of valuable genes has been tapped for improving legumes. Conventional breeding (selection from landraces and primitive cultivars, pedigree, bulk, backcross, or single-seed descent methods of selection), mutation breeding, exploitation of somaclonal variability, and genetic transformation have helped breeders to select superior cultivars of grain legumes. Commercial hybrid production using cytoplasmic male sterility (CMS) is a success story for pigeonpea, where hybrids produced a 4 to 52% increase in yield over the parents. This is feasible because the natural out-crossing in pigeonpea ranges from 20 to 40% (see Chapter 4). Faba bean is also a partially (34%) allogamous crop and cross-pollination ranges from 4% (practically a selfer) to 84% (practically an outcrosser) (see Chapter 6). The major obstacle in producing hybrid legumes is the structure of the flower, which ensures a 99% chance of self-pollination in most grain legumes. Lentil contains small cleistogamous flowers, making it virtually 100% self-pollinating (see Chapter 8). Outcrossing in mungbean is only 0.5 to 3% (see Chapter 10).

1.5.1 Breeding for Plant Type

Breeders have developed determinate semidwarf and dwarf plant types with uniform maturity for common bean, pea, cowpea, faba bean, pigeonpea, lupin, mungbean, and azuki bean by conventional breeding. Semidwarf varieties with determinate plant type are resistant to lodging and therefore adapted to mechanical harvesting. Early maturing (less than 98 days), high-yielding common bean varieties with upright growth habit can be machine harvested, which is cost-effective for common bean growers (see Chapter 2). A major breakthrough in pea came about when breeders combined reduced crop height (e.g., *le*) and conversion of leaflets to tendrils (e.g., *af*), described as the semidwarf, semileafless ideotype. The semidwarf and semileafless types provided a number of

benefits, such as reduced leafiness and excessive overshading, increased aeration and reduced disease in some environments, and improved ease of harvest of both garden and field pea types as a consequence of reduced lodging (see Chapter 3). An artificially induced mutant for determinate growth in faba bean resulted in a cultivar that facilitated easy machine harvesting (see Chapter 6). Dwarfing genes have been identified in pigeonpea and are being used to develop dwarf cultivars. Most pigeonpea varieties at reproductive stage achieve the height of 2 to 3 m. The dwarf-inbred lines range in height from 70 to 80 cm and produce reasonable yields (see Chapter 4).

1.5.2 Breeding for High Yield

Substantial gain in yield has been achieved in all grain legumes through innovative conventional breeding, but it is still far behind that of cereals. Conventional breeding produced high-yielding cultivars containing genes for resistance to biotic (fungal diseases, viruses, and pests) and abiotic (cold, heat, drought, adverse soil nutrition, and lodging) stresses. High-yielding pigeonpea varieties have been produced by mutation breeding (see Chapter 4). A somaclonal variant in pigeonpea also produces white seeds, with a 25% increase in seed size and a 30% advantage in yield (see Chapter 4). Several national and international laboratories have developed transformation techniques to incorporate genes for resistance to pests and pathogens.

1.5.3 Breeding for Canning Quality

Major advances in breeding of major grain legumes include production of varieties for canning, revolutionizing the canning industries. Major market classes of common bean include bayo, great northern, “ojo de cabra” (creamed-striped), pinto, pink, and red Mexican beans. Dark red kidney bean cultivar “Montcalm” with excellent canning quality, has been developed by conventional breeding (see Chapter 2). Immature faba bean seeds are canned (usually precooked) and frozen. The canned and lightly precooked “baby” types (less than 12 mm in length) have a very high price in Spain (José Cubero, personal communication, May 26, 2004).

1.5.4 Breeding for High Protein

Grain legumes are a rich source of protein (see Table 1.1). They are used for feeding animals, as well as for human consumption as *dhal*, or soup. Protein content in pigeonpea ranges from 20 to 22%. Its wild relatives have a protein content of up to 32% from which lines with high protein content have been developed (see Chapter 4). Faba bean usually has a 25 to 28% protein content, with high lysine. Successful breeding programs have released some faba bean cultivars with as much as 32 to 35% protein (see Chapter 6). Protein content in lupins ranges from 32.1 to 44%. It has been suggested that three to four genes control protein content, and the action of these genes is additive and complementary (see Chapter 9).

1.5.5 Breeding for Vegetable Type

Vegetable-type legumes, particularly common bean, pea, cowpea, pigeonpea, faba bean, and chickpea, command high market prices. Snap bean cultivars with green pods are known as French, garden, green, or stringless beans. Snap bean with flat or cylindrical pods, yellow (waxy type), green, or purple colors, and long or short pods are used for fresh, frozen, and canning purposes (see Chapter 2). Cowpea with edible pods are widely grown in various Asian and Pacific countries and IITA has developed vegetable-type cowpea varieties (see Chapter 5). ICRISAT has released several vegetable-type pigeonpea cultivars. One such variety, ICPL 87079, is highly popular in India, Africa, and China. Green pigeonpea is an important vegetable in the Caribbean (see Chapter 4). Immature-type pea is used to produce canned or frozen products (see Chapter 3).

1.5.6 Breeding for Antinutritional Elements

Grain legumes contain numerous antinutritional elements that reduce the biological value of protein and are harmful to humans and animals if consumed raw. Common bean without phytohemagglutinin has been produced by backcrossing (Bollini et al., 1999). Consumption of common bean helps reduce cholesterol and cancer risk (see Chapter 2). A large fraction (50%) of seed protein in common bean constitutes the phaseolin and lectin-related protein family. Phytohemagglutinin and lectin-related proteins in bean seeds are toxic to monogastric animals. Cowpea and pigeonpea contain trypsin and chymotrypsin inhibitors and tannins, and these elements can be eliminated by heat treatment and by varietal improvement. However, cowpea is a rich source of calcium, iron, and zinc, which is desirable from a nutritional standpoint. These elements may increase the seed hardness that requires longer cooking time. Soaking of the seeds before cooking reduces the cooking time (see Chapter 5). Faba bean contains tannins, vicine, convicine, and low glycosides related to favism, which causes strong stomach hemorrhaging (see Chapter 6). Faba bean is rich in 3, 4-dihidroxiphenilalanine (L-DOPA, which is used against Parkinson's disease and has a potential pharmaceutical use). Breeding methods combined with mechanical processing have reduced these antinutritional elements in faba bean. The development of lupin as a modern crop began with the selection of plants with reduced alkaloid content in seeds (see Chapter 9). Wild relatives of cultigens often do not contain antinutritional factors that could be introgressed into cultivars when a wide hybridization technique is developed. Transformation may also play a role in producing grain legumes without antinutritional elements. Several laboratories worldwide are engaged in producing transgenic legumes.

1.6 CONCLUSIONS

1. Grain legumes (rich in protein) and cereals (rich sources of carbohydrate) are an excellent combination for a balanced human diet. Crop rotation of legumes with cereals enriches the soil because legumes fix nitrogen in symbiotic association with the *Rhyzobium* species.
2. Grain legume breeders have confined their efforts to the primary gene pool (GP-1). Exploitation of the secondary (GP-2) and tertiary (GP-3) gene pools is hampered because of pre- and postzygotic barriers. The national and international research institutes have conducted extensive plant exploration to collect primitive cultivars, landraces, and wild relatives before the spread of highyielding varieties and environmental factors make them extinct. These invaluable materials are being deposited in gene banks for medium- and long-term storage. GP-3 has not been identified for pea and faba bean.
3. Breeders have achieved substantial yield gain by conventional breeding by producing varieties that are resistant to abiotic and biotic stresses. However, the yield of grain legumes is lower than that of cereals on a per-hectare basis.
4. Breeders have produced varieties—in some grain legume crops—with high protein content and without antinutritional elements.

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CHAPTER 2
Common Bean (Phaseolus vulgaris L.)

Shree P. Singh

2.1 INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the most important of over 50 *Phaseolus* species native to the Americas, occupying more than 85% of areas sown to these species worldwide. This chapter describes different types of common bean, their usage, production, and production constraints. This chapter will also cover organization of genetic diversity, strategies used for integrated genetic improvement, progress achieved through breeding, and future prospects. Since 1999, I have edited a book (Singh, 1999d) and workshop proceedings (Singh, 2000), and written a review article (Singh, 2001a) and a book chapter (Singh, 2001b). Thus, an additional exhaustive review of literature is not justified at the moment. Only a few pertinent publications will be cited wherever necessary. Readers should refer to other literature for details.

2.1.1 Snap Bean

There are two major types of common bean: snap and dry. Snap bean cultivars for green-pod harvest are also called garden, green, or stringless bean. Fully developed green pods of snap bean have reduced fiber in the pod walls and sutures. Both determinate bush and indeterminate climbing snap bean cultivars exist. The latter permit multiple harvests over a longer period of time and yield much higher per unit area of cropped land than their bush counterparts. The cultivation of climbing snap bean cultivars is more popular in China, home gardens in Europe, winter sowings in Florida, and around larger cosmopolitan cities in Latin America and elsewhere.

There has been a major effort in Europe and the U.S. to develop snap bean cultivars within the last 75 years. Large variation in plant type, fruiting pattern, maturity, and the length, shape, color, fleshiness, and other pod characteristics of snap bean cultivars have resulted. Snap bean cultivars with flat or cylindrical pods, yellow (waxy types), green, or purple colors, and long or short pods are used fresh, frozen, or in cans. Cylindrical types exist in a range of diameters, with the largest common in the U.S. and the smallest in Belgium and France. The U.S., Europe, and China are the largest producers of snap bean. Although the exact area of snap bean cultivars planted is not known, it is estimated to be less than 3 million hectares.

Most snap bean breeding is private. Improved germplasm and information are often proprietary and not readily available. Among the public institutions in the U.S., Oregon State University at Corvallis has the most active breeding program, especially for the blue lake types (Myers, 2000; Myers and Baggett, 1999). Breeding and genetics of snap bean are also carried out at Cornell University, Geneva, New York (Phillip Griffiths, personal communication, October 2003) and the University of Wisconsin, Madison (James Neinhuis, personal communication, October 2003). Resistance to Fusarium root rot [caused by *Fusarium solani* f. sp. *phaseoli* (Burk.) Snyder & Hansen] was bred at USDA-ARS, Prosser, Washington (Silbernagel, 1987). Stavely and McMillan (1992) developed rust [caused by *Uromyces appendiculatus* (Pers.:Pers.) Ung.] resistant snap bean germplasm. McMillan et al. (1998) developed climbing snap bean germplasm resistant to Bean golden yellow mosaic virus (BGYMV, a geminivirus) using marker-assisted selection. Davis and Myers (2002) and Skroch and Nienhuis (1995) reported patterns of genetic diversity among snap bean cultivars that may have a broad genetic base compared to dry bean. For example, 'Oregon 91G' has "S" Mesoamerican phaseolin, while its morphological and horticultural characters are of the Andean bean (Myers, 2000). For decades, breeders in the U.S. and Europe have crossed extensively to Middle American germplasm to introgress disease resistance, small seed and pod size, and other traits. Major improvements include a change from climbing to bush growth habit, increased lodging resistance, and concentration of pod set. Similarly, improved pod characteristics include stringless or low pod fiber, round pod cross-section, straight and smooth pods, darker green interior and exterior color, reduced interlocular cavitation, slow seed development, and incorporation of pod pubescence (Myers, 2000; Myers and Baggett, 1999). Resistance to anthracnose [caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. & Cav.], *Bean common mosaic virus* (BCMV, a potyvirus), Bean common mosaic necrosis virus (BCMNV, a potyvirus), bacterial brown spot (caused by *Pseudomonas syringae* pv. *syringae* van Hall), common bacterial blight [caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye], halo blight [caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkh.)], cucumber mosaic virus, Beet curly top virus (BCTV, a curtovirus), rust, white mold [caused by *Sclerotinia sclerotiorum* (Lib) de Bary], and against various root rot complexes have also been incorporated singly or in combination. In a few cases, resistance to heat, cold, and ozone were improved. Nienhuis and Sass (1999) provide a comprehensive list of snap bean cultivars released.

Maximizing pod yield and quality of the determinate growth habit Type I cultivars destined for a single, destructive harvest offers a daunting challenge to breeders. Moreover, cultivars adapted to low-input organic and conventional farming systems, and possessing resistance to major abiotic and biotic stresses will be required. For details regarding snap bean, readers should refer to the literature cited above. The remainder of the discussion in this chapter will refer to dry bean.

2.1.2 Dry Bean

The leading use of common bean is as dry seed. Consumer preferences for dry bean size, color, shape, and brilliance vary a great deal (Singh, 1992; Voysest, 2000). Based on seed size, dry bean is commonly grouped into small (<25 g 100 seeds^{-1}), medium (25 to 40 g 100 seeds^{-1}), and large (>40 g 100 seeds^{-1}) seed classes. In Brazil, Venezuela, and

Central America, small-seeded dry bean of different colors (predominantly black, cream, cream-striped, or red) is preferred. Similarly, medium-seeded “bayo” (beige), pinto (cream spotted), and “flor de mayo” (pink speckled) are preferred in the central highlands of Mexico. In Canada and the U.S., pinto, large red kidney, and small white (navy or pea) beans are preferred. In the Andes of South America, the Caribbean, Africa, Asia, and Europe, large-seeded dry bean of various colors (except black) is popular. In Latin America, the highest per capita consumption of dry bean is in Brazil and Mexico (>10 kg per year). In Rwanda and Burundi, per capita consumption is more than 40 kg per year. Dry, green-shelled, and snap bean have high nutritional value, especially in conjunction with cereals and other carbohydrate-rich foods; beans reduce cholesterol and cancer risks (Andersen et al., 1984; Myers, 2000). Dry bean dishes range from beans simply boiled in water to more sophisticated preparations of baked beans, cakes, chips, creams, pastes, salads, soups, and stews (Hosfield et al., 2000).

Dry bean cultivars harvested for green-shelled bean are often large-seeded cream-mottled (known as barbunya, borlotti, cacahuate, cargamanto, cavalo, cranberry, frutilla, chitti, and speckled or Natal sugar), pink-mottled, purple-mottled, red-mottled, or white-mottled. The distinguishing characteristic of such cultivars is that the pods change color (turn red or purple, with or without stripes) when fresh seed is ready to be harvested for consumption. The pods not removed for green-shelled seeds are allowed to mature normally on the plant to be harvested later as dry bean. The natives in the highlands of Peru and Bolivia grow popping dry bean, known as nuñas. In East Africa, cooked young green leaves are consumed.

From its origin and domestication regions in the Andean South and Middle America, common bean production and consumption has expanded into other parts of the Americas (from about 35°S to >50°N latitude and from sea level to >3,000 m altitude) (Gepts et al., 1988; Singh, 1992). Similar dissemination to Africa, Asia, Europe, and other parts of the world occurred within the last five centuries (Gepts and Bliss, 1988). Dry bean is grown annually on more than 14 million hectares worldwide (Singh, 1999a). The Americas are the largest dry-bean-producing regions (6.7 million MT), and Brazil (2.5 million MT) is the largest producer and consumer in the world. Asia (2.2 million MT), Africa (2.1 million MT), and Europe (1 million MT) follow the lead of the Americas in dry bean production.

The U.S. (1.3 million MT) and Mexico (0.98 million MT) follow Brazil as leading dry bean producers. Argentina, Canada, Colombia, Nicaragua, Honduras, Guatemala, El Salvador, Peru, Haiti, Ecuador, Chile, Cuba, Venezuela, and the Dominican Republic are also important dry-bean-producing countries in the Americas. Production has increased substantially in the last 50 years in Argentina, Bolivia, Brazil, Canada, and the U.S., largely due to the increase in area planted in these countries. Among Asian countries, China (1.3 million ha), Iran, Japan, and Turkey are the major producers of dry bean. In Africa, Burundi, Ethiopia, Malawi, Republic of South Africa, Rwanda, Tanzania, Uganda, and Zimbabwe form the list of important dry-bean-producing nations. In recent years, it is becoming evident from the production statistics that in Europe (and Asia), Albania, Belarus, Bulgaria, Croatia, Greece, Italy, Moldova Republic, Poland, Romania, Spain, Ukraine, and Yugoslavia are the major dry bean producers.

Common bean is a short-day crop (White and Laing, 1989). Mildly cool environments favor growth and development. Below 2,000 m elevation in tropical and subtropical Latin

America, dry bean is grown twice during the year, often coinciding with the prevalent bimodal rainfall patterns of these regions (March to June and September to December). Thus, in environments with 16 to 18°C mean growing temperatures, with about 12-hour days, and free from abiotic and biotic stresses, most cultivars complete their growing cycle from germination to seed maturity in 90 to 120 days. However, it is common to harvest dry bean in 60 to 80 days in environments with mean growing temperatures of 20 to 24°C, such as those occurring in Valle del Cauca, Colombia. In the highlands (above 2,000 m elevation) of Bolivia, Colombia, Ecuador, and Peru, climbing cultivars often require more than 250 days to mature (planted in October and November, harvested in May and June). In the humid highlands of Guatemala and Mexico, and in Principado de Asturias, Spain, climbing cultivars require approximately 150 days to maturity.

Most cultivars grown in the highlands of Mexico, Central America, and the Andes are highly sensitive to photoperiod, and will not complete their growing cycle under long summer days at higher latitudes (>14-hour days) in the U.S., Canada, Europe, and Japan. Cultivars adapted to higher latitudes have either evolved after their dissemination from the primary centers of domestication in the Andes, Central America, and Mexico (e.g., Anasazi, Common Great Northern, Common Pinto, Common Red Mexican, San Juan, and Local Pink in the U.S.), or have been developed by breeding (discussed later). Although two major independent alleles control sensitivity to photoperiod (Gu et al., 1998), genetic control of adaptation to higher latitude and phenological differences among supposedly photoperiod-insensitive cultivars of the same growth habit and market class grown in North America are not fully understood.

While rainfed cultivation occurs in Argentina, Brazil, the Andes, Central America, highlands of Mexico, southwestern Canada, and in the northeastern and midwestern U.S., especially in areas with more than 400 mm rainfall, dry bean might require supplemental irrigation for secured harvest and higher yield. In most of Europe, western Asia, April to July wintercrop in central Brazil, Chile, the Pacific coast of Peru and Mexico, and California, Idaho, Oregon, Washington, and Wyoming, irrigation is an absolute requirement. In regions with warm or hot summers, dry bean is grown in the autumn (e.g., northeastern Argentina), spring (Indo-Gangetic plains of India), or winter (in Brazil and many countries in Africa and the Caribbean).

Determinacy is controlled by a single recessive allele and is associated with bush plant type, although a few determinate climbers are known to occur. There is continuous variation in growth habit in indeterminate cultivars, from bush to extreme climbing types. Singh (1982b), however, classified growth habits into four major classes using the type of terminal bud (vegetative vs. reproductive), stem strength (weak vs. strong), climbing ability (nonclimber vs. strong climber), and fruiting patterns (mostly basal vs. along entire stem length or only in the upper part). These are the Type I=determinate bush, Type II=indeterminate upright bush, Type III=indeterminate, prostrate, nonclimbing or semiclimbing, and Type IV=indeterminate, strong climbers.

At higher latitudes in temperate climates, dry-bean cultivars of growth habit Types I, II, and III predominate. These are harvested within 90 to 120 days from planting. Cultivars of growth habit Types I, II, and III are grown in monoculture as well as under different relay, strip, and intercropping systems throughout the world (Singh, 1992). Type IV cultivars always require support. Thus, these are grown either in association with maize (*Zea mays* L.), cassava (*Manihot esculenta* Crantz), coffee (*Coffea arabica* L.),

sugarcane (*Saccharum officinarum* L.), and other crops, or they are grown on trellises or stakes. Type IV cultivars are popular in several regions throughout the world, such as Principado de Asturias, Spain, highlands of the Andes, humid highlands of Central America and Mexico, and Florida in the U.S. In some of these regions, highly priced snap or dry bean cultivars are grown for higher yields and multiple harvests. Although intercropping is often more profitable (Francis and Sanders, 1978) and may be favored for sustainable farming, yield reductions with intercropping and large genotype×cropping system interactions occur for cultivars of all growth habits (Clark and Francis, 1985).

Although dry bean is grown in a wide range of soil types, light loamy soils with pH between 5.5 to 7.0 and rich in organic matter are more suitable for good bean production. A 100- to 120-day crop with a yield of 2,500 kg ha^{-1} will usually remove 60 to 80 kg of soil nitrogen and 40 kg of phosphorus. Acidic soils often are deficient in nitrogen and phosphorus and contain toxic levels of aluminum or manganese. Similarly, in somewhat alkaline soils, deficiency of microelements (e.g., zinc, iron, and boron) is common. Thus, it is essential to use appropriate corrective measures. These measures include the adequate use of lime, gypsum, sulfur, manure or composted manure, and fertilizers rich in nitrogen and phosphorus, as well as other major and minor elements (Fageria et al., 1995; Tarkalson et al., 1998; Thung and Rao, 1999). Moreover, in most traditional bean-growing regions, nodulation and nitrogen fixation are common, although not adequate for high yields. Use of inoculants with the most effective and competitive Rhizobium strains may be promoted, while simultaneously minimizing use of nitrogenous fertilizers at the time of sowing or restricting to foliar applications only when necessary.

Both abiotic and biotic stresses limit dry bean production (Schwartz and Pastor-Corrales, 1989; Wortmann et al., 1998). Low soil fertility (Thung and Rao, 1999), as noted earlier, and drought stress (Acosta-Gallegos and Kohashi-Shibata, 1989; Terán and Singh, 2002a) are widespread production constraints. Drought is frequent in northeastern Brazil, coastal Peru, the central and northern highlands of Mexico, and the western U.S. Complete crop failures under rainfed or dryland conditions are not uncommon in these areas. In regions where the crop is planted toward the end of the rainy season (e.g., September to December in Central America), moderate drought stress also frequently occurs.

High temperatures (>30°C day or 20°C night) during flowering, especially when relative humidity is low, can severely limit bean production. Recurring low temperatures (below 10°C), as well as frost in the highlands (above 2,000 m elevation) of Latin America and in the U.S. and Canada during the growing season, can reduce yield and quality up to 100%.

Common bacterial blight is a widespread problem from tropical to temperate dry-bean-growing environments. In relatively cooler and wetter areas, halo blight and bacterial brown spot may cause severe losses of yield and quality. Angular leaf spot, anthracnose, and rust are considered among the most widely distributed foliar fungal diseases that cause severe yield losses in Latin America, Africa, and other parts of the world. Various root rots (Abawi, 1989) in most dry bean-growing environments, web blight [caused by *Thanatephorus cucumeris* (Frank) Donk.] in warm and wet Latin American environments, and ascochyta blight [caused by *Phoma exigua* var. *diversispora* (Bub.) Boerma] in cool and wet African and Latin American environments occasionally become severe. Similarly, in the U.S. and Canada, white mold is endemic in most

production regions. BCMV and BCMNV in dry bean production regions worldwide, Bean golden mosaic virus (BGMV, a geminivirus) in Argentina, Bolivia, and Brazil, and BGYMV in tropical and subtropical Central America, coastal Mexico, the Caribbean, and the southeastern U.S. cause severe yield losses. BCTV in the northwestern U.S. and bean yellow mosaic virus in the same region and in some European countries, the Middle East, North Africa, and Asia can also cause severe yield losses.

Leafhoppers (*Empoasca kraemeri* Ross and Moore) (in the tropics and subtropics) and *E. fabae* Harris (in temperate and cooler environments) are the most widely distributed insect pest in dry bean fields, especially in low rainfall areas. Bean pod weevil (*Apion godmani* Wagner and *A. aurichalceum* Wagner) causes severe damage to pods and seeds in the highlands of Mexico, and in Guatemala, El Salvador, Honduras, and Nicaragua (Garza et al., 1996, 2001). In the highlands of Mexico and in the U.S., the Mexican bean beetle (*Epilachna varivestis* Mulsant) causes severe leaf damage, especially in late maturing cultivars. The bean fly (*Ophiomyia phaseoli* Tryon) is by far the most damaging insect in Africa (Abate and Ampofo, 1996; Wortmann et al., 1998). The bean weevil *Zabrotes subfasciatus* Boheman (in warm tropical and subtropical environments) and *Acanthoscelides obtectus* (Say) (in cool and temperate environments) cause severe damage to seed for consumption or planting when dry bean is not properly stored.

Many broadleaf and grassy weeds invade dry bean fields (Waters and Morishita, 2000). The composition of weed population and the most dominant weed species vary from region to region and depend upon several factors. These factors include the growing environments (dry vs. wet, or warm vs. cool), agronomic management of both the standing dry bean and other crops grown on the farm, and the history of the fields being used for production. Other factors that influence weed population and composition are the cropping and tillage systems, growth habit and competitive ability of cultivars, planting density, moisture availability, and pest control measures.

2.2 ORGANIZATION OF GENETIC DIVERSITY

2.2.1 Phaseolus Species in Relation to the Common Bean

Debouck (1991, 1999) and Debouck and Smartt (1995) discussed the taxonomy and phylogenetic relationship among *Phaseolus* species in relation to the common bean. Freytag and Debouck (2002) described in considerable detail the taxonomy, distribution, and ecology of the genus *Phaseolus* in North America, Mexico, and Central America. However, genetic diversity among *Phaseolus* species is organized into primary, secondary, and tertiary gene pools, based on the ability to cross with the common bean. The primary gene pool of each species comprises both the wild populations (i.e., the immediate ancestor of cultivars) and cultivars. *P. coccineus* L. (scarlet runner), *P. costaricensis* Freytag & Debouck, and *P. polyanthus* Greenman (synonymous with *P. dumosus*, year-long bean) form the secondary gene pool. The tertiary gene pool comprises *P. acutifolius* A. Gray (tepary bean) and *P. parvifolius* Freytag. Lima bean (*P. lunatus* L.) and other species compose the quaternary gene pool. However, in addition to the common bean, only Lima, scarlet runner, tepary, and year-long bean are cultivated.

2.2.2 Cytology and Cytogenetics

Cultivars and wild populations of most *Phaseolus* species, including *P. vulgaris*, contain 2n=2x=22 chromosomes. Debouck (1999) provided the number of available accessions for some species and reviewed their useful traits and the extent of chromosome pairing between interspecific hybrids. Despite the fact that the common bean chromosomes are extremely small, Mok and Mok (1977) at the prophase of somatic cells, and Cheng and Bassett (1981) at the diplotene stage of meiosis in pollen mother cells, identified all 11 chromosomes. Ashraf and Bassett (1986) induced translocations. Ashraf and Bassett (1987) also reported five primary trisomics, four tertiary trisomics, and two tetrasomics. Thus far, this knowledge has not facilitated gene mapping and introgression.

2.2.3 Linkage Maps

Until the early 1990s, our knowledge regarding the number of alleles and quantitative trait loci (QTL) controlling different traits and linkage among them, length of different linkage groups, and their association with the 11 chromosomes was fragmentary and limited to a few plant morphological, seed, and isozyme traits (Bassett, 1991). Availability of DNA-based markers has aroused interest and greatly facilitated development of linkage maps within the last 15 years. For example, Kelly et al. (2003) listed 14 common bean populations that have been used to develop linkage maps since 1992. However, use of different size and type of population (e.g., F_2, backcross, recombinant inbred lines), marker types (e.g., RFLP, RAPD, SCAR, SSR), and lack of coordination among researchers have often hindered and slowed the pace of development of fully saturated integrated linkage maps. Gepts (1999) reported the total map length in common bean of approximately 1200 cM. Blair et al. (2003) developed a genome-wide anchored microsatellite map of common bean with a total map length of 1720 cM and average chromosome length of 156.4 cM. Moreover, all 11 expected linkage groups have been identified with their respective chromosomes using the fluorescent *in situ* hybridization (Pedrosa et al., 2003), and distribution of favorable alleles and QTL on the 11 linkage groups summarized (Kelly et al., 2003). For additional information on linkage maps, readers should refer to the literature cited and to Adam-Blondon et al. (1994), Freyre et al. (1998), Miklas et al. (2002a), Nodari et al. (1993a), Tar'an et al. (2002), and Vallejos et al. (1992).

2.3 DOMESTICATION, FLORAL BIOLOGY, AND GENETIC VARIATION IN COMMON BEAN

Wild populations of common bean are distributed from Chihuahua in northern Mexico to San Luis, in northeastern Argentina (Gepts et al., 1986). Common bean is a noncentric crop. Multiple domestications occurred throughout the distribution range of its wild populations in Mexico, Central America, and Andean South America (Gepts et al., 1986).

Common bean cultivars and wild populations have cleistogamous papilionacious flowers that are highly self-pollinated (<1% outcrossing). Nonetheless, Ibarra-Pérez et al. (1997) reported outcrossing rates ranging from 0 to 78% for individual families with mean rate for six dry bean genotypes ranging from 4.4 to 10.2% in California. Anthesis

occurs in early morning hours, and crosses are made with or without emasculation of anthers prior to anthesis. Hybrids between wild and cultivated common bean are fully fertile and no known barriers exist for gene introgression and exchange (Singh et al., 1995a).

During domestication, common bean evolved from extreme indeterminate climbing to determinate bush types, from sensitivity to insensitivity to long photoperiod, and from small- to large-seeded forms. Similarly, it has evolved from having impermeable to water-permeable seed coat, and from types that shatter due to highly fibrous pod walls to forms with less fiber that are less subject to shattering (Gepts, 1998; Gepts and Debouck, 1991). Major alleles and QTL that influenced common bean domestication have been identified (Koinange et al., 1996) and mapped (Freyre et al., 1998; Gepts, 1999). These traits are growth habit *(fin)*, photoperiod insensitivity *(ppd, hr)*, pod fiber *(St)*, seed dormancy, and seed weight. Existence of a considerably larger variation in phaseolin types, an evolutionary marker (Gepts, 1988), in wild bean populations compared to cultivars suggests that not all wild beans were domesticated, and cultivars may have reduced genetic diversity (Gepts, 1998; Koenig et al., 1990).

Unequivocal evidence of existence of the large-seeded Andean and small- and medium-seeded Middle American common bean gene pool was provided by (1) establishing the relationship between seed size, *Dl-1* vs. *Dl-2* alleles (Shii et al., 1980), and F_1 hybrid incompatibility (Gepts and Bliss, 1985; Singh and Gutiérrez, 1984); (2) phaseolin seed proteins (Gepts et al., 1986); (3) allozymes (Singh et al., 1991c); (4) morphological traits (Singh et al., 1991b); and (5) DNA markers (Becerra-Velásquez and Gepts, 1994; Haley et al., 1994c). Singh (1989) described in considerable detail the patterns of variation among common bean cultivars. Moreover, based on morphological, biochemical, adaptive, and agronomic traits, and geographical distribution in their primary centers of origin and domestication, Singh et al. (1991a) further divided the Andean and Middle American gene pool into six races: three Andean gene pool (all large-seeded)=Chile, Nueva Granada, and Peru races; and three Middle American gene pool=Durango (medium-seeded semiclimber), Jalisco (medium-seeded climber), and Mesoamerica (all small-seeded) races. Beebe et al. (2000) reported the existence of additional diversity within the Middle American gene pool, especially a group of Guatemalan climbing bean accessions that were distinct from previously defined races. For details regarding characteristics of races, readers should refer to the literature cited.

Cultivars of races Durango, Mesoamerica, and Nueva Granada are planted in more than 80% of the area under dry bean worldwide. Cultivation of Jalisco race is restricted to the highlands of Mexico, and Peru race is limited to the Andean highlands. Some favorable alleles and QTL found in races, gene pool, and wild common bean populations, and secondary and tertiary gene pool and their introgression into dry bean cultivars will be discussed later.

2.4 INTEGRATED GENETIC IMPROVEMENT

As discussed later, high levels of resistance to anthracnose, BCMV, BCMNV, and rust are found in dry bean cultivars. However, available resistance is inadequate for angular leaf spot, ascochyta blight, bacterial brown spot, bean fly, bruchids, common bacterial

blight, halo blight, leafhoppers, web blight, white mold, and against most root rot complexes. Despite the fact that limited evaluations have been done for these stresses, alien germplasm seems promising for cultivar improvement (see Singh, 2001b). For example, resistance to bruchids was found in wild *P. vulgaris* (Acosta-Gallegos et al., 1998; van Schoonhoven et al., 1983), *P. acutifolius* (Shade et al., 1987), and also other *Phaseolus* species (Dobie et al., 1990). *Phaseolus polyanthus* is particularly known for its resistance to angular leaf spot (Mahuku et al., 2003a), anthracnose (Mahuku et al., 2003b), and ascochyta blight (Schmit and Baudoin, 1992). *P. coccineus* has long been known as a source of resistance to angular leaf spot (Busogoro et al., 1999; Mahuku et al., 2003a), anthracnose (Hubbeling, 1957; Mahuku et al., 2003b), common bacterial blight (Schuster et al., 1983; Singh and Muñoz, 1999), and white mold (Abawi et al., 1978; Gilmore et al., 2002). Thus, favorable alleles and QTL are scattered across cultivated and wild populations in the primary, secondary, and tertiary gene pool of common bean. Moreover, only a small portion of the available genetic diversity has been used globally for common bean improvement (Miklas, 2000; Singh, 1992).

Analysis of the pedigrees of dry bean cultivars released around the world shows that the genetic basis of cultivars within each market class is extremely narrow (McClean et al., 1993; Miklas, 2000; Voysest et al., 1994). Nonetheless, in dry bean, large differences in seed characteristics (e.g., size, shape, and color), growth habit, and adaptation traits of cultivars occur. Moreover, there are some problems of recombination (Johnson and Gepts, 1999; Mumba and Galwey, 1999; Singh and Gutiérrez, 1984; Singh and Molina, 1996) between races and gene pools. It would be impossible to accumulate at once or in a single attempt all the necessary favorable alleles and QTL from across races and gene pools of the common bean, and its wild populations and related species into one high-yielding cultivar resistant to major abiotic and biotic stresses. Thus, a two- to three-tiered breeding strategy (Kelly et al., 1998a; Singh, 2001a; White and Singh, 1991) is often used for integrated genetic improvement. For discussions regarding breeding history, see Adams (1996), Dean (2000), Kelly (1999, 2001), and Voysest (2000), and for different selection methods used, refer to Fouilloux and Bannerot (1988), Kelly et al. (1998b), and Singh (1992, 1999b, 2001a).

For an integrated genetic improvement to develop broadly adapted high-yielding cultivars that are less dependent on water, fertilizers, pesticides, and labor, I shall discuss: (1) introgression of favorable alleles and QTL from alien germplasm, (2) pyramiding favorable alleles and QTL for improvement of specific traits, and (3) simultaneous improvement of multiple traits for cultivar development.

2.4.1 Introgression of Favorable Alleles and QTL from Alien Germplasm

The knowledge of genotype performance per se, combining ability for quantitative characters such as seed yield and drought resistance, occurrence of incompatibility alleles, and undesirable linkages is essential to successful introgression of favorable alleles and QTL from alien germplasm. The problem of introgression and recombination often gets magnified as the genetic distance increases between the cultivar to be improved and alien germplasm. Because of the differences in genetic distance and the breeding methods and strategies that are required, introgression of favorable alleles and

QTL from each alien market class, race, gene pool, wild population, and related species from the secondary and tertiary gene pool may need to be accomplished separately. These introgressions will be briefly discussed here.

2.4.1.1 Introgression from the Tertiary Gene Pool

Phaseolus acutifolius (tepary) is native to northwestern Mexico and the southwestern U.S. High level of resistance to ashy stem blight and Fusarium wilt (Miklas et al., 1998b), bruchids (Dobie et al., 1990; Shade et al., 1987), common bacterial blight (Schuster et al., 1983; Singh and Muñoz, 1999), drought (Federici et al., 1990; Markhart, 1985), and leafhoppers (Cardona and Kornegay, 1999) occur in some accessions of tepary bean. Miklas and Santiago (1996) also reported some resistance to BGYMV, and Miklas and Stavely (1998) found incomplete dominance for rust resistance.

To cross *P. vulgaris* with *P. acutifolius*, the F_1 hybrid embryos must be rescued between two and three weeks after pollination (Haghighi and Ascher, 1988; Mejía-Jiménez et al., 1994; Thomas and Waines, 1984). Moreover, one or more backcrosses to the recurrent common bean parent are essential because the interspecific hybrids are male sterile (5 to 8 bivalent chromosome pairings occur at metaphase I). Using *P. acutifolius* as the female parent of the initial F_1 cross or the first backcrossing of the *P. vulgaris*×*P. acutifolius* hybrid on to *P. acutifolius* is often more difficult than using *P. vulgaris* as the female parent of the initial cross and first backcrossing the interspecies hybrid on to *P. vulgaris* (Mejía-Jiménez et al., 1994). Haghighi and Ascher (1988) and Mejía-Jiménez et al. (1994) demonstrated the usefulness of congruity backcrossing over recurrent backcrossing for recovery of fertility and high number of hybrid progenies between interspecific crosses of common and tepary bean. Muñoz et al. (2004) reported an increased percentage introgression (8.8%) of amplified fragment length polymorphism bands in congruity backcross-derived interspecific breeding lines compared with those derived from a single backcross (5.2%). Nonetheless, the usefulness of congruity backcrossing for introgression of favorable alleles and QTL is not known, because none of the former were resistant to common bacterial blight (S.Singh and C.G.Mufioz, unpublished results), whereas resistant breeding lines were selected from the latter group (Singh and Muñoz, 1999).

Tepary bean possesses the highest level of resistance to common bacterial blight, while only moderate resistance occurs in the common bean (Schuster et al., 1983; Singh and Muñoz, 1999). Resistance to common bacterial blight in tepary bean is controlled by independent dominant alleles (Freytag, 1989; Urrea et al., 1999). Urrea et al. (1999) also reported quantitative inheritance of common bacterial blight resistance. McElroy (1985), using interspecific populations developed at the University of California, Riverside (Thomas and Waines, 1984), Scott and Michaels (1992), and Singh and Muñoz (1999) transferred moderate level of common bacterial blight resistance from the tepary to common bean.

2.4.1.2 Introgression from the Secondary Gene Pool

The natural habitat for *P. polyanthus* is the highlands of Guatemala, and that for *P. coccineus* is the highlands of Mexico. As the name suggests, *P. costaricensis* was

recently discovered in the highlands of Costa Rica (Freytag and Debouck, 1996). As noted earlier, some accessions of *P. coccineus* and *P. polyanthus* have a high level of resistance to angular leaf spot, anthracnose, ascochyta blight, and white mold. Unlike common bean and species in the tertiary gene pool, the members of the secondary gene pool are characterized by a significant amount of outcrossing (from 20 to 40%). The three species cross among themselves and each is crossed with the common bean without embryo rescue (10.84 to 10.98 bivalent chromosome pairings at metaphase I), particularly when common bean is used as the female parent (Manshardt and Bassett, 1984; Park and Dhanvantari, 1987; Singh et al., 1997). However, hybrids between crosses of common bean and any of the three species may be partially sterile, and it may be difficult to recover the desired stable common bean phenotypes (Wall, 1970).

Researchers have successfully used backcrossing to the recurrent common bean parent for introgression of a moderate level of resistance to common bacterial blight from *P. coccineus* (Freytag et al., 1982; Miklas et al., 1994a and 1994b; Park and Dhanvantari, 1987). Although resistance to white mold in *P. vulgaris*×*P. coccineus* populations was reported to be controlled by a dominant allele (Abawi et al., 1978; Schwartz et al., 2004), only an intermediate level of resistance was introgressed into common bean (Miklas et al., 1998a). It is likely that either *P. coccineus* used in interspecific hybridization did not have the highest level of white mold resistance or inadequate disease screening, and selection methods were used.

2.4.1.3 Introgression from Wild Common Bean

Mahuku et al. (2003a) reported angular leaf spot resistance in some wild common bean populations that has yet to be introgressed into and pyramided with the resistance already found in Andean and Middle American cultivars (discussed later). On the other hand, resistance to *Z. subfasciatus* was not found in thousands of common bean cultivars screened, and only a few wild beans from the highlands of Mexico were resistant (van Schoonhoven et al., 1983). Arcelin present as the main storage protein in the cotyledons of wild bean was responsible for the resistance (Osborn et al., 1988). There are more than six dominant alleles at the arcelin locus with varying effects (Acosta-Gallegos et al., 1998; Cardona et al., 1990; Kornegay et al., 1993). The presence of arcelin can easily be detected by SDS-PAGE electrophoresis. Because antiserum to the protein is available, it is easier, faster, and cheaper to use the ELISA technique for large germplasm screening. This development has facilitated and expedited breeding for bruchid resistance and minimized dependence on screening using insect infestation that is time-consuming and affected by the environment. It should be noted that arcelin, like lectin and other related proteins, is heat labile, and hence its antinutritional effects are lost during prolonged (>30 minutes) cooking.

Except for the occasional occurrence of *Dl-1* and *Dl-2* alleles in Andean×Middle American wild bean crosses (Koinange and Gepts, 1992), there are no known barriers for transferring favorable alleles from wild populations of either gene pool into cultivars. The F_1 hybrids between cultivated×wild, as well as their progenies in subsequent generations, are fully fertile. Such crosses have been used to study inheritance of seed size and determine yield potential (Singh et al., 1995a) and to study genetic differences between the wild and cultivated phenotypes (Koinange et al., 1996).

Only a small number of alleles with large phenotypic effects, clustered in a few regions of the genome, control the inheritance of traits involved in domestication (Koinange et al., 1996). It is therefore not difficult to recover the cultivated phenotype from wild×cultivated crosses. For example, a recurrent backcross program was used to introgress an extremely high level of resistance to bruchids into a range of cultivars (Cardona et al., 1989, 1990; Kornegay and Cardona, 1991). On the contrary, a more elaborate germplasm conversion program and molecular markers tightly linked with major genes involved in domestication may be required to convert desired wild beans to realize their full agronomic potential.

2.4.1.4 Introgression between and within Cultivated Common Bean Gene Pool

Readers interested in specific examples of introgression of favorable alleles and QTL between market classes, races, and gene pools of common bean cultivars should refer to Beaver (1999), Brick and Grafton (1999), Kelly (2001), Miklas (2000), and Singh (2001a). Only general strategies used and problems encountered will be briefly discussed here. Moreover, sometimes within cultivars introgression and pyramiding of favorable alleles can be achieved simultaneously. Therefore, progress achieved in improvement of specific traits will be discussed later. From bi-parental Andean by Middle American inter-gene pool crosses it is often difficult to recover essential agronomic characteristics of either parent, irrespective of the conventional pedigree, bulk-pedigree, and singleseed-descent selection methods used (Johnson and Gepts, 1999; Kornegay et al., 1992; Welsh et al., 1995). More elaborate programs of recurrent or congruity inbred-backcrossing (Urrea and Singh, 1995) and recurrent selection (Beaver and Kelly, 1994; Kelly and Adams, 1987) are required. Moreover, there may be an occasional need for the use of bridging-parents if *Dl-1* and *Dl-2* incompatibility alleles occur between the Andean and Middle American germplasm to be hybridized (Singh and Gutiérrez, 1984). In general, complementation and positive combining ability for seed yield (Singh et al., 1992b, 1993), and resistance to drought stress (White et al., 1994b) occurs between different races within the Middle American gene pool. Thus, it is not too difficult to introgress and pyramid favorable alleles and QTL among races Durango, Jalisco, and Mesoamerica, especially when the differences in phenological and seed traits are not large. However, greater than 75% genetic contribution of the cultivar under improvement must be ensured for easier recovery of its desirable attributes. Moreover, three-way or modified-double crosses (Singh, 1982a), recurrent or congruity inbred-backcrosses (Bliss, 1993; Urrea and Singh, 1995), or recurrent selection (Beaver and Kelly, 1994; Kelly and Adams, 1987; Singh et al., 1999) are used to recover desirable attributes.

2.4.2 Pyramiding of Favorable Alleles and QTL for Improvement of Specific Traits

2.4.2.1 Growth Habit and Upright Plant Type

Popular cultivars and landraces of black, cream, cream-striped, red, and other seed colors of race Mesoamerica in the tropics and subtropics have a prostrate semiclimbing growth

habit Type III (Singh, 1989; Singh et al., 1991a). However, a few black-seeded landraces such as Jamapa, Porrillo Sintetico, Rio Tibagi, San Fernando, and Tacaragua with tall indeterminate upright stem and branches of growth habit Type II exist. Traditional race Durango cultivars of great northern, pink, pinto, and red seed types also possess a growth habit Type III. In comparatively humid environments, such cultivars are prone to anthracnose, web blight, white mold, and seed discoloration because of their closed canopy and pods touching the ground. Upright cultivars that permit better air movement through the canopy and keep pods away from the soil are preferred (Coyne et al., 1974; Park, 1993; Schwartz et al., 1987). These traits were introgressed from Type II race Mesoamerica into race Durango cultivars using phenotypic recurrent selection (Kelly and Adams, 1987) and other breeding methods (Coyne et al., 2000; Kelly et al., 1999d). Recently developed cultivars also carry resistance to BCMV and rust.

A recessive allele *fin* controls inheritance of determinate growth habit Type I (Bliss, 1971). Type I cultivars are less stable (Ghaderi et al., 1982; Kelly et al., 1987) and low yielding (Nienhuis and Singh, 1985). Adams (1982) and Grafton et al. (1993) used Type II cultivars to change Type I growth habit of navy and small white cultivars into more stable high-yielding Type II. Similarly, cream-striped carioca beans (traditionally a Type III) with growth habit Type II and resistance to leafhopper and five diseases were developed using gamete selection (Singh et al., 1998, 2000c). Singh et al. (2003a) improved stem stiffness of a small-seeded black bean germplasm line A 55 that had a tall (>70 cm plant height) growth habit Type Ila, and reduced branches. Kelly (2001) provided an excellent review of variation in growth habit or plant type. He also reviewed historical development and germplasm and strategies used for breeding and remaking of plant architecture for efficient production and high yield for different market classes of dry bean.

2.4.2.2 Seed Yield

Differences in yield potential are much larger between, rather than within, market classes, races, and gene pools (Singh, 1989; Singh et al., 1991a). Adams (1982), Kelly (2001), Kelly et al. (1998b), Singh (1991, 1992), and Wallace et al. (1993) discussed strategies for breeding for higher bean yields. Heritability of yield varied from low to moderately high (Singh et al., 1991e; Welsh et al., 1995).

High-yielding genotypes have been developed using mass-pedigree (Singh et al., 1989a, 1993) and recurrent (Singh et al., 1999) selection methods from interracial populations within the Middle American gene pool. Negative associations between seed size and yield (White and González, 1990; White et al., 1992) limited progress by Kornegay et al. (1992) and Singh et al. (1989b). Nonetheless, Beaver and Kelly (1994) and Singh et al. (1999, 2002) selected large-seeded high-yielding genotypes from Andean×Middle American populations using recurrent selection.

2.4.2.3 Biological Nitrogen Fixation

Nodulation and N_2-fixation by *Rhizobium* species are evaluated in terms of number and weight of nodules, level of acetylene reduction activity, and amount of total fixed nitrogen in seed and other plant parts. The latter uses a ^{15}N-isotope dilution method.

Differences in foliage color, vigor, total dry matter, and seed yield of uninoculated and inoculated plots are very useful practical measures of biological nitrogen fixation. Graham (1981) reviewed problems of nodulation and N_2-fixation in common bean. N_2-fixation depends upon bacterial strains, common bean genotypes, and soil moisture and fertility, especially nitrogen and phosphorus levels. Nodulation and N_2-fixation are favored by cool long growing season (such as those occurring in Latin American highlands and at higher latitudes) and by growing late maturing cultivars of indeterminate growth habit (taking >100 days to maturity) in absence of water stress in soils deficient in nitrogen and rich in phosphorus and other essential nutrients (Castellanos et al., 1996; Pereira and Bliss, 1987).

Large genotypic differences occur for nodulation and N_2-fixation (Hardarson et al., 1993; Pereira et al., 1989). Broad-sense heritability for the level of acetylene reduction activity ranged from 0.25 to 0.71 (McFerson, 1983), and for seed nitrogen content ranged from 0.47 to 0.73 (Miranda and Bliss, 1987). Asokan (1981) found a single dominant allele determining low nitrogenase activity and ineffective nodulation.

Bliss (1993) discussed strategies for improving N_2-fixation. Pereira et al. (1993) reported 'Puebla 152', U.W. 22–34, and BAT 76 genotypes as possessing a positive general combining ability for number of nodules. They increased nodule number and weight after three cycles of recurrent selection. Bliss et al. (1989) developed five high N_2-fixing genotypes. Given the increasing emphasis on low-input sustainable agriculture and need for reducing dependence on chemical fertilizers, breeding for high N_2-fixation should be a priority.

2.4.2.4 Drought Resistance

Drought is among the most widely distributed and endemic production problems in many regions of the world. Drought is severe in northeastern Brazil, coastal Peru, the central and northern highlands of Mexico, and in the western U.S. (Acosta et al., 1999; Terán and Singh, 2002a). Drought adversely affects all plant parts and reduces seed yield and quality (Acosta-Gallegos and Adams, 1991; Acosta-Gallegos and Kohashi-Shibata, 1989). Abebe et al. (1998), Acosta et al. (1999), and Terán and Singh (2002a) reported large genotypic differences for drought resistance in dry bean. Cultivars from race Durango had the highest level of drought resistance (Terán and Singh, 2002a), as would be expected because of their origin and domestication in semiarid Mexican highlands (Singh, 1989; Singh et al., 1991a). Among physiological and agronomic traits, mean seed yield (the arithmetic and geometric means) of drought-stressed (DS) vs. nonstressed (NS) environments was found to be the most effective selection criterion (Ramirez-Vallejo and Kelly, 1998; White et al., 1994a). Narrow-sense heritability for seed yield in DS ranged from 0.09 to 0.80 (Schneider et al., 1997b; Singh, 1995; White et al., 1994b). Schneider et al. (1997a) reported four RAPD markers in one bi-parental population, and five in another that were consistently associated with DS yield, NS yield, or geometric mean (GM) yield in DS and NS environments. They concluded that the effectiveness of marker-assisted selection for drought resistance was inversely proportional to heritability of yield in DS environment.

Given the expense involved, large quantities of seed required, and relatively small gains obtained, the early generation selection for drought resistance may not be justified

(Terán and Singh, 2002b). In contrast, by evaluating recombinant-inbred-lines, Rosales-Serna et al. (2000) and Schneider et al. (1997a, 1997b) developed drought-resistant breeding lines from bi-parental populations using seed yield or RAPD markers as selection criteria. Singh (1995) advanced doublecross interracial and inter-gene pool populations from F_2 to F_5 using a single-pod-bulk method, and then developed F_5-derived F_7 recombinant inbred lines, all in NS environment, for subsequent evaluation in replicated trials in DS and NS environments. He used the arithmetic mean seed yield of DS and NS environments and percent reduction in yield due to drought stress as selection criteria, and identified significantly higher yielding drought-resistant genotypes. Still higher levels of drought resistance should be expected from pyramiding favorable alleles and QTL from recently identified germplasm in races Durango, Jalisco, and Mesoamerica (Terán and Singh, 2002a). Moreover, the highest level of drought resistance reported in *P. acutifolius* (Federici et al., 1990; Markhart, 1985) has yet to be introgressed into and combined with the highest level of resistance available in common bean.

2.4.2.5 Heat Resistance

As noted earlier, common bean production is favored in environments with 16 to 18°C mean growing temperatures. High temperatures (>30°C day or >20°C night) during anthesis and seed set in tropical lowlands (below 600 m elevation), and during summer at higher latitudes (e.g., California, Colorado, Idaho, Nebraska, Washington, and Wyoming), especially when relative humidity is low, can severely limit common bean production. Moreover, high night temperature seems to have more adverse effects than high day temperature. Reduced pollen viability and pollen tube growth (Haterlein et al., 1980; Weaver et al., 1985); excessive abortion of flowers and young pods; reduced seed set per pod, seed size, and yield (Dickson and Petzoldt, 1989; Shonnard and Gepts, 1994) are the more conspicuous effects of prolonged high temperature in common bean. Carbohydrate partitioning and biological nitrogen fixation are adversely affected after heat stress. Photoperiod sensitivity alleles are also known to interact with high temperatures to exacerbate photoperiod sensitivity such that in tropical lowlands, highly sensitive genotypes may develop elongated internodes, remain vegetative, and not flower at all.

Bouwkamp and Summers (1982) reported that a single dominant or two complementary dominant alleles controlled resistance to high temperature-drought stress in common bean. Shonnard and Gepts (1994) also documented that determinacy (*fin* gene), or a factor tightly linked to it, induced susceptibility to heat in the Central Valley of California. However, quantitative inheritance with low to moderate heritability has also been found (Roman and Beaver, 2001; Shonnard and Gepts, 1994). In tropical and subtropical Latin America, often the small-seeded dry bean cultivars of race Mesoamerica seem to have higher level of resistance to high temperatures. Small black 'Negro Argel' and medium-seeded G 5273 exhibited heat resistance in California (P.Gepts, personal communication, May 2004). Also, a few large-seeded Andean dry and snap bean genotypes have exhibited heat resistance in the U.S. (Dickson and Petzoldt, 1989; Rainey and Griffiths, 2004; Shonnard and Gepts, 1994) and Puerto Rico (Baiges et al., 1996). Whether these possess complementary mechanisms and alleles for high temperature resistance should be worth investigating. Moreover, breeders interested in improving high temperature resistance should screen a much wider range of germplasm for parental selection and crossing, and delay screening for heat resistance until the latter generations. Furthermore, it is important to accurately define the actual heat stress affecting the crop. For example, is the heat stress accompanied by drought stress? What growth stage or tissue is affected? Researchers should realize that not all heat stresses are alike. It is also important to determine whether the plant is able to recuperate after a heat stress by producing an additional flux of flowers, pods, and seeds. While this may partially offset yield losses, it may increase the crop duration and interfere with harvest.

2.4.2.6 Low Soil Fertility Resistance

Common bean is susceptible to deficiencies or toxicities of soil minerals (Thung and Rao, 1999; Wortmann et al., 1998). Calcareous soils of the Pacific Northwest U.S. have excess minerals such as calcium, magnesium, potassium, and sodium. Consequently the high

soil pH (>7.0) induces iron, manganese, phosphorus, or zinc (Zn) deficiencies (Westermann and Singh, 2000). Polson and Adams (1970) in Michigan, and Moraghan and Grafton (1999) in North Dakota reported a widespread Zn deficiency. Zn deficiency also triggered iron deficiency in zinc-inefficient cultivars (Jolley and Brown, 1991).

General symptoms of mineral deficiency or toxicity may include poor emergence; slow growth; seedling and adult plant stunting; leaf yellowing, chlorosis, and bronzing; early seedling death; reduced overall growth and dry matter production; delayed and prolonged flowering and maturity; excessive flower and pod abortion; low harvest index; reduced seed weight; deformed and discolored seeds; and up to 100% yield loss. Root growth may also be adversely affected (Cumming et al., 1992; Fawole et al., 1982a).

Root development under phosphorus deficiency in common bean is genetically controlled (Fawole et al., 1982a). Genetic variation for tolerance to phosphorus (Thung, 1990; Yan et al., 1995a, 1995b), Zn (Polson and Adams, 1970; Westermann and Singh, 2000), and iron (Zaiter et al., 1987) deficiencies were also reported. Similarly, tolerance to aluminum toxicity was documented (Foy et al., 1972; Noble et al., 1985). Quantitative inheritance with low to intermediate heritability for ability to absorb or utilize, and for tolerance to low phosphorous was reported (Fawole et al., 1982b; Lindgren et al., 1977; Urrea and Singh, 1989). In contrast, Singh and Westermann (2002) found a single dominant allele *Znd* in great northern 'Matterhorn' controlling resistance to soil Zn deficiency. Forster et al. (2002) also reported a single dominant allele that controlled seed-Zn accumulation in the efficient navy bean 'Voyager'. The monogenic dominant control of these traits should facilitate their transfer into needing cultivars. Tolerance to phosphorus deficiency and response was introgressed from tropical to temperate germplasm using inbred-backcrossing (Schettini et al., 1987).

Singh et al. (2003c) and Wortmann et al. (1995) for germplasm screening, Urrea and Singh (1989) for studying inheritance, and Singh et al. (1989a, 1989b) for selection studies, all applied multiple deficient or toxic mineral stresses. Tolerance to such low soil fertility was found in all three Middle American races (Singh et al., 2003c). Contrary to the earlier reports by Lynch and Beebe (1995) and Yan et al. (1995a, 1995b), large-seeded Andean germplasm had much lower level of tolerance. Moreover, breeding lines such as A 321, A 445, and A 744 with low soil fertility tolerance, were developed from interracial populations within the Middle American gene pool (Singh et al., 1989a, 2003c), but not from intra-racial populations (Singh et al., 1989b).

2.4.2.7 Angular Leaf Spot Resistance

High level of resistance (but not immunity) to the Andean and Middle American races of *P. griseola* causing angular leaf spot was reported (Guzmán et al., 1995; Pastor-Corrales et al., 1998). The resistance is controlled by a dominant allele (Singh and Saini, 1980). Ferreira et al. (2000) found a single dominant allele in breeding line MAR 2 (Singh et al., 2003b), imparting resistance to the race 63.39 of *P. griseola* that was linked in coupling phase with a RAPD marker at 5.8 cM distance. Correa et al. (2001) found a dominant resistant allele to the same pathogen race in 'Ouro Negro', but a recessive resistance allele to *P. griseola* race 31.23 in pinto 'UI 111'. Nietsche et al. (2000) also reported a dominant allele controlling resistance to the pathogen race 31.17 in small black bean Cornell 49–24–2. Moreover, the same RAPD marker was linked with the resistance

found in MAR 2 and Mexico 54, thus suggesting that all three common bean genotypes probably possessed the same resistance allele, *Phg 2*. Caixeta et al. (2003) reported a dominant allele for resistance to race 61.41 in dry bean breeding line BAT 332 that was linked to two RAPD markers in sis-position.

de Oliveira et al. (2002) followed the introgression of angular leaf spot resistance alleles into inbred-backcross lines derived from crosses of 'Ruda' with MAR 2 and Mexico 54. Singh et al. (2003b) developed resistant germplasm such as A 339, MAR 1, MAR 2, and MAR 3 from interracial populations between the three Middle American races. Nonetheless, favorable alleles found in Nueva Granada (e.g., G 5686, G 19833, Jalo EEP 558) and other Andean races and *P. coccineus* and *P. polyanthus* (Mahuku et al., 2003a) also need to be combined to increase the level of angular leaf spot resistance and broaden the genetic base of cultivars. Breeders and pathologists should also keep in mind that angular leaf spot incidence and severity increases with the plant age such that genotypes exhibiting high level of resistance in early growth stages can actually be susceptible during flowering and pod maturation. Genetically broad-based extremely high level of resistance during the entire crop duration is essential for the hotspots such as central and northeastern Brazil, where angular spot is often very severe and pathogenic variability is high. Thus, greenhouse screening in the vegetative stage against a few races or isolates may not suffice.

2.4.2.8 Anthracnose Resistance

Collitotrichum lindemuthianum, the cause of anthracnose, is highly variable (Balardin et al., 1997). Like *P. griseola*, *C. lindemuthianum* co-evolved with common bean (Melotto et al., 2000; Pastor-Corrales et al., 1995; Sicard et al., 1997) and both Andean and Middle American pathogen groups occur. High level of resistance to anthracnose occurs in common bean (Pastor-Corrales et al., 1995) and *P. coccineus* and *P. polyanthus* (Mahuku et al., 2003b). Kelly and Vallejo (2004) have thoroughly reviewed the genetics of anthracnose resistance, including the map location, linked markers, and breeding value of major resistance alleles. In brief, eight dominant (*Co-1* to *Co-7* and *Co-9* and *Co-10*, of which *Co-3* and *Co-9* are allelic) and one recessive *(Co-8)* alleles control resistance. Of these, only *Co-1* is of Andean origin, and all others occur in Middle American germplasm. Multiple allelic series occur at the *Co-1*, *Co-3*, and *Co-4* loci. Moreover, molecular markers are available for all resistance alleles except *Co-3* and *Co-7*. Readers interested in details should refer to Kelly and Vallejo (2004) and the extensive literature cited by them.

Kelly et al. (1994) developed the anthracnose-resistant black-seeded cultivar Raven, which was then used to develop resistant 'Phantom' (Kelly et al., 2000). Miklas et al. (2003b), using marker-assisted selection, introgressed the *Co-4*^{2} resistance allele into a pinto breeding line, USPT-ANT-1. The *Co-4*^{2} is one of the three resistance alleles found in Mexican landrace Colorado de Teopisca (synonymous with G 2333) (Young et al., 1998). Kelly et al. (1999b) also combined the Andean *Co-1* and Middle American *Co-2* alleles for anthracnose resistance in a large-seeded light red kidney bean 'Chinook 2000'. Both resistance alleles were also combined in small black cultivar Jaguar (Kelly et al., 2001).

Singh et al. (2003b) developed breeding lines that, in addition to angular leaf spot resistance, also have broad-based pyramided anthracnose resistance from races Durango (Guanajuato 31 synonymous with G 2618), Jalisco (Ecuador 299 synonymous with G 5653), and Mesoamerica (PI 207262 synonymous with G 1320). Use of molecular markers should facilitate introgression and pyramiding of favorable alleles. In addition, resistance from *P. coccineus* and other species needs to be utilized.

2.4.2.9 BCMV and BCMNV Resistance

Both viral diseases are aphid-, mechanically, and seed-transmitted (Morales and Castaño, 1987), and cause severe yield losses in susceptible cultivars worldwide. The BCMNV strains cause necrosis of aerial plant parts, especially at higher temperatures (Drijfhout et al., 1978). Both strain-specific and nonspecific resistance to BCMV and BCMNV are found (Drijfhout, 1978; Drijfhout et al., 1978). Also, molecular markers for the dominant *I* (Haley et al., 1994a; Melotto et al., 1996) and recessive *bc-1*^{2} (Miklas et al., 2000) and *bc-3* (Haley et al., 1994b; Johnson et al., 1997) resistance alleles are available.

The *I* resistance allele was introgressed into great northern (Coyne et al., 2000; Kelly et al., 1999d; Myers et al., 2001b), 'flor de mayo' (Acosta-Gallegos et al., 1995a), and pinto (Brick et al., 2001; Myers et al., 2001a) genotypes. Kelly et al. (1995) discussed strategies for pyramiding BCMV and BCMNV resistance alleles. Resistance alleles *I* and *bc-1*^{2} have been combined into pinto 'Kodiak' (Kelly et al., 1999a) and great northern UI98–209G (Stewart-Williams et al., 2003). Pinto germplasm 92US-1006 carries *I* and *bc-2*^{2} (Silbernagel, 1994). Through an exceptional nationwide informal collaborative effort, pathologists and breeders have pyramided *I* and *bc-3* resistance alleles, and combined them with rust resistance into great northern BelMiNeb-RMR-6 to 13 and pinto BelDakMi-RMR-14 to 23 beans (Pastor-Corrales, 2003; Pastor-Corrales et al., 2001). Miklas and Kelly (2002) developed cranberry and Miklas et al. (2002b) developed light and dark red and white kidney bean germplasm lines resistant to BCMV and BCMNV. In addition to molecular markers, a clearer understanding of the evolutionary origin (Andean vs. Middle American) of the *I* resistance allele and its undesirable linkage with the *B* allele for seed coat color (Kyle and Dickson, 1988; Temple and Morales, 1986) should facilitate breeding. The alternate linkage of *b* and *I* alleles is available in landrace San Cristobal 83 from the Dominican Republic and breeding lines BAT 1235, CRAN 028, PVA 800A, and UI 51.

2.4.2.10 BGMV and BGYMV Resistance

The separation of BGMV, occurring in Argentina, Brazil, and Bolivia from BGYMV that predominates in Mexico, Central America, the Caribbean, and southern U.S. occurred in 1998 (see Garrido-Ramirez et al., 2000) although molecular diversity between the two groups was reported earlier (Gilbertson et al., 1991). Nonetheless, resistance to both viruses can be expressed as percent infection, plant dwarfing, leaf chlorosis or yellowing, and pod deformation (Morales and Niessen, 1988). Resistance to each of these component traits is controlled by a different allele (Molina Castañeda and Beaver, 1998; Velez et al., 1998). In contrast, Morales and Singh (1991) reported a quantitative inheritance for combined symptom expression. There are four or more sources of

resistance to leaf chlorosis. One of the sources found in race Durango (e.g., landrace cultivar 'Garrapato' synonymous with G 2402) was inadvertently transferred into breeding line A 429 (Morales and Singh, 1993). This resistance is controlled by the recessive allele *bgm-1* (Blair and Beaver, 1993; Urrea et al., 1996; Velez et al., 1998). The recessive resistance allele bgm-2 to leaf chlorosis, first reported in breeding line DOR 303 by Velez et al. (1998), is probably of Andean origin. Resistance to leaf chlorosis found in small-seeded black landraces such as Porillo Sintetico and Turrialba and present in 'DOR 390', 'Dorado' (synonymous with DOR 364), and 'ICA Pijao' controlled by a major QTL (Miklas et al., 1996) is nonallelic to those present in A 429 and DOR 303. Osorno et al. (2003) reported two additional recessive alleles imparting resistance to leaf chlorosis in *P. coccineus* accession G 35172. None of the five recessive alleles alone imparts complete resistance to leaf chlorosis under severe disease pressure. However, the *bgm-1* seems to have the largest effect, and it complements the effects of the QTL present in small black bean landraces. Breeders interested in the highest level of resistance, especially for South America, should test the usefulness of pyramiding these alleles in different combinations, and combine them systematically with dominant alleles imparting resistance to plant dwarfing (Blair et al., 1993) and pod deformation (Molina Castañeda and Beaver, 1998). The recessive resistance allele *bgm-1* from A 429 has been used to develop highly resistant small red (e.g., DOR 482, MD 30–75, Tio Canela 75), black (e.g., Turbo III), and carioca bean (Singh et al., 1998, 2000c) cultivars for Central America and Brazil, using pedigree, mass-pedigree, or gamete selection methods. Beaver et al. (1999) were the first to develop BGYMV (*bgm-1* allele), common bacterial blight, and rust resistant large-seeded light red kidney bean breeding line PR9443–4 for the Caribbean. Moreover, availability of a RAPD and SCAR marker (Urrea et al., 1996) has minimized the need for and complemented disease screening in presence of the virus. For example, introgression of the *bgm-1* resistance allele from A 429 into snap bean cultivars for the southeastern U.S. was accomplished using the RAPD marker (McMillan et al., 1998). Singh et al. (2000a, 2000b) pyramided high level of resistance in different dry beans using direct screening that was subsequently verified by the presence of molecular markers.

2.4.2.11 Common Bacterial Blight Resistance

Resistance found in common bean is controlled by one major QTL (Ariyarathne et al., 1999; Miklas et al., 1996) and that which is introgressed from tepary bean is determined by two major QTL (Pedraza et al., 1997; Yu et al., 2000). In addition, five to eight QTL with small effects determine inheritance of common bacterial blight resistance (Jung et al., 1996; Nodari et al., 1993b). Although resistance found in scarlet runner bean (Freytag et al., 1982; Miklas et al., 1994b; Park and Dhanvantari, 1987) has been introgressed, its inheritance and complementation to the common or tepary bean resistance is not known.

The initial pyramiding of common bacterial blight resistance was carried out by S. Temple, S. Beebe, and M. Pastor-Corrales at the Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. Also, R.E.Wilkinson at Cornell University, Ithaca, New York (with partial funding and germplasm from CIAT, and working with researchers from Mayaguez, Puerto Rico) participated in that work. The resulting "XAN" and "Wilk" breeding lines were developed at CIAT. Singh and Muñoz (1999), while

introgressing common bacterial blight resistance from the tepary bean (VAX 1 and VAX 2), also combined with the XAN and Wilk germplasm to develop subsequent breeding lines (VAX 3 to VAX 6) possessing the highest level of pyramided common bacterial blight resistance (also see Singh et al., 2001). Nonetheless, there are tepary accessions (e.g., G 40029, G 40156) that possess much higher levels of resistance than that introgressed and pyramided to date (Singh and Muñoz, 1999). More systematic introgression and pyramiding of common bacterial blight resistance are therefore warranted.

The SU91 (Pedraza et al., 1997) and BC420 (Yu et al., 2000) SCAR markers are tightly linked with independent common bacterial blight resistance QTL in the common bean breeding line XAN 159. XAN 159 derives its resistance from tepary PI 319443 (McElroy, 1985). Scott and Michaels (1992) did not observe any segregation for common bacterial blight reaction in tepary PI 319443/PI 440795 F_2 population. The QTL introgressed in OAC 88–1 from tepary PI 440795 by Scott and Michaels (1992) and in VAX 1 and VAX 2 by Singh and Muñoz (1999) from tepary G 40001 is also present in XAN 159 (P.N. Miklas, personal communication, May 2004). Thus, only two independent major QTL imparting common bacterial blight resistance have been introgressed from tepary bean to date. Furthermore, while more than 80% of *P. acutifolius* cultivars were highly resistant to common bacterial blight, fewer than 25% of wild accessions possessed similar resistance (CIAT, 1996). The former, therefore, may have a narrower genetic base and the same resistance alleles and QTL might be present in multiple cultivated tepary. Researchers interested in introgressing additional resistance from tepary to common bean should fingerprint unknown accessions or perform allelism tests with those used thus far, prior to interspecific hybridization, to identify different complementary resistance alleles and QTL for introgression.

2.4.2.12 Halo Blight Resistance

Resistance to halo bacterial blight is inherited by a single dominant or recessive allele (Asensio et al., 1993; Taylor et al., 1978). Race-specific and nonspecific resistance to halo blight in common bean is found. For example, GN Nebraska #1 Sel. 27 and PI 150414 carry non-race-specific resistance (Taylor et al., 1978). GN Nebraska #1 Sel. 27 (Coyne et al., 1967) and PI 150414 (Hagedorn et al., 1974) were used for improving halo blight resistance. Small white bean cultivar Edmund also carries high level of resistance. Ariyarathne et al. (1999) mapped halo blight resistance QTL whose usefulness still needs to be verified in independent populations for marker-assisted selection. Moreover, a systematic effort to search for additional sources of resistance and pyramiding in addition to those already known is long overdue.

2.4.2.13 Root Rot Resistance

Several root rots adversely affect dry bean production worldwide (Abawi and Pastor-Corrales, 1990). Although two or more fungi-causing root rots often occur together and are endemic, their composition and relative importance may vary from year to year and region to region. Moreover, severity and incidence of root rots may be spotty within a field. Among the root rots of major economic importance are Fusarium root rot (caused

by *Fusarium solani* f.sp. *phaseoli*), Fusarium wilt or yellows (caused by *Fusarium oxysporum* f.sp. *phaseoli*), Rhizoctonia root rot (caused by *Rhizoctonia solani*), charcoal rot or ashy stem blight (caused by *Macrophomina phaseolina*), and southern blight (caused by *Sclerotium rolfsii*). Pythium root rot (caused by *Pythium* spp.), black root rot (caused by *Thielaviopsis basicola*), and Aphanomyces root-and-hypocotyl rot are of local or regional importance. Continual bean cropping, high soil compaction, water stress, inadequate drainage, low organic matter, and high and low temperatures may accentuate root rot problems and change composition of the root rot complex. For example, charcoal rot often dominates the root rot complex in warm dry climates such as those occurring in northeastern Brazil. In relatively cool and wet regions such as Michigan, Minnesota, and Wisconsin, *F. solani* f.sp. *phaseoli* may be more prevalent.

Some studies of pathogenic variability [e.g., for *F. oxysporum* f.sp. *phaseoli; M. phaseolina*] and germplasm screening and identification of root rot-resistant germplasm have been carried out. For example, Cramer et al. (2003) and Salgado and Schwartz (1993) characterized *F. oxysporum* f.sp. *phaseoli* variability using differential cultivars or RAPD markers. Abawi and Pastor-Corrales (1990), Beebe et al. (1981), and Tu and Park (1993), among others, reported root rot-resistant common bean germplasm. Schneider and Kelly (2000) developed a reliable greenhouse screening protocol for Fusarium root rot, and reported 99% correlation between the greenhouse and field evaluations using a diverse group of genotypes. In their tests, medium- and large-seeded cultivars [except snap bean breeding line FR 266 that derives resistance from Mexican black bean landrace N203 (synonymous with PI 203958)] were relatively more susceptible than small-seeded counterparts. Resistance to Fusarium root rot in FR 266 was polygenically controlled (h^2 ranging from 48 to 71%) and strongly influenced by environments (Schneider et al., 2001). The RAPD markers (except one) associated with greenhouse screening tended not to associate with field screening, and individual markers did not explain more than 15% of variation for resistance.

Salgado et al. (1995) found a single dominant, a recessive, or duplicate recessive resistance to a Colorado race of *F. oxysporum* f.sp. *phaseoli*. Cross et al. (2000) reported a single dominant resistance allele for race 4 of the pathogen in medium-seeded race Durango cultivars. But the resistance was polygenically controlled in small-seeded race Mesoamerica cultivars with estimated heritability of 0.85 and realized heritability ranging from 0.25 to 0.60. Fall et al. (2001) identified and mapped a major QTL on the linkage group (LG) 10 from small black breeding line A 55 that controlled 63.5% of variance for *F. oxysporum* f.sp. *phaseoli* resistance.

Two independent complementary dominant alleles (*Mp-1* and *Mp-2*) controlled resistance to charcoal rot in drought-resistant dry bean breeding line BAT 477, and RAPD markers for one allele in coupling and for the other allele in repulsion phase were identified (Olaya et al., 1996). Mayek-Pérez et al. (2001) also reported a similar inheritance of resistance in BAT 477. However, from a field screening of 119 $F_{5:7}$ recombinant inbred lines from 'Dorado'/XAN 176 population, Miklas et al. (1998c) reported a quantitative inheritance (h^2 0.53 and 0.57) for charcoal rot with RAPD markers linked to four QTL (each controlling 13 to 19% of variation for resistance) in 1993 and three QTL in 1994. Silbernagel et al. (1998) developed a BCMV and root rot-resistant pinto bean breeding line, USWA-20. In general, difficulty of screening segregating populations, early generation families, and breeding lines on a large scale have hindered a

more concerted long-term breeding effort for resistance to the individual and collective diseases known as root rots.

2.4.2.14 Rust Resistance

One of the most variable pathogens of common bean is *U. appendiculatus*, the cause of rust (Stavely, 1984). It is wind borne and is thus highly mobile and possesses great potential for changing its population composition within and between growing seasons and production regions. Fortunately, from judicious field screening over the years through international nurseries and screening in greenhouses with known races (Stavely, 1984), high level of resistance has been identified in both Andean and Middle American cultivars. While the genetics of resistance found in some important sources of germplasm are still not fully understood, nearly a dozen different alleles carrying race-specific resistance have been identified (Grafton et al., 1985; Stavely, 1990). Boone et al. (1999), Haley et al. (1993), Johnson et al. (1995), Jung et al. (1996), and Miklas et al. (1993) reported RAPD or SCAR markers for incorporating rust resistance into cultivars. These markers enabled placement of many of the resistance alleles on the integrated linkage map (Miklas et al., 2002a). These markers could also be used to combine rust resistance with resistance to other diseases. For example, Pastor-Corrales (2003) and Pastor-Corrales et al. (2001) reported breeding lines (developed by Stavely and his collaborators) of great northern, pinto, and other market classes with pyramided resistance to BCMV, BCMNV, and rust. This kind of germplasm that combines alleles from both Andean and Middle American gene pools, and confers resistance to the broadest spectrum of pathogenic races in the U.S., could be combined further with the presence of trichomes on abaxial leaf surfaces imparting race nonspecific resistance (Shaik, 1984). In the face of the potential for genetic variability of this pathogen has been the amazing durability of *Ur-3* resistance allele in the temperate production areas of North America, but this success could come to an abrupt end.

2.4.2.15 White Mold Resistance

White mold is a major concern to common bean growers throughout North America. It also can be a severe problem in Argentina and southern Brazil. Crop losses can reach 90% in the central high plains (Kerr et al., 1978; Schwartz et al., 1987). High humidity and temperatures of 10 to 25°C enhance disease development (Weiss et al., 1980). The lack of cultivars with high level of resistance has hampered our understanding of variation in virulence among isolates of this pathogen. Moreover, environmental effects and disease avoidance mechanisms often dwarf the genetic differences for physiological resistance.

Common bean germplasm with only partial physiological resistance to white mold have been reported (Middleton et al., 1995; Miklas et al., 1999). Plant architectural traits that impart upright growth habit and porous canopy help reduce white mold incidence and severity (Kolkman and Kelly, 2002; Park, 1993). The highest levels of physiological resistance occur in *P. coccineus* (Abawi et al., 1978; Gilmore et al., 2002). However, resistance in common bean has low heritability (Genchev and Kiryakov, 2002; Park et al., 2001). On the other hand, Miklas and Grafton (1992) and Miklas et al. (2001, 2003a)

reported moderate to high heritability. Abawi et al. (1978) and Schwartz et al. (2004) reported a single dominant allele controlling resistance in *P. vulgaris*×*P. coccineus* populations. Park et al. (2001) reported RAPD markers linked with as many as nine QTL responsible for white mold resistance in 'PC 50'/XAN 159 common bean population. Most markers linked to field resistance were located on the LG B4, B7, B8, and B11, with the QTL on B7 accounting for the largest (12%) variation in resistance. Miklas et al. (2001) identified one AFLP marker linked with a QTL for the greenhouse straw test (mapped on LG B7) in the A 55/G 122 population. The same and one additional QTL for physiological resistance in the field and another QTL responsible for canopy porosity (mapped on LG B1) was also identified.

Kolkman and Kelly (2003) reported white mold resistance QTL from 'ICA Bunsi'/'Newport' population that were located on LG B2 and B7. They used recombinant inbred lines from 'Huron'/Newport population to confirm linkage between markers and QTL derived from ICA Bunsi (synonymous with Ex-Rico 23) for white mold resistance across environments. Miklas et al. (2003a) identified two QTL for white mold resistance in 'Benton'/NY 6020–4 snap bean population. The QTL on LG B6 derived from Benton explained 12% of the variation for resistance whereas the QTL from NY 6020–4, responsible for 38% variation, was located on LG B8. The latter was associated with increased internode length. Thus, QTL for white mold resistance have been located on 7 of the 11 LG. Because most QTL individually have small to moderate effects on resistance and have not been confirmed in independent populations, it would be worth investigating the comparative usefulness of marker-assisted versus direct disease screening in the greenhouse and field for introgression and pyramiding of white mold resistance.

Lyons et al. (1987) carried out recurrent selection for white mold resistance in interspecific populations with some success. However, examples of cultivars with physiological resistance are very few, especially among great northern, pink, pinto, and red market classes; pinto 'Chase' has intermediate resistance against white mold (Coyne et al., 1994). New resistant germplasm such as I 9365–3, I 9365–5, I 9365–31, and 92BG-7 (Miklas et al., 1998a) derived from *P. vulgaris*×*P. coccineus* populations have been more effective than *P. vulgaris* sources in multilocation tests (Steadman et al., 2001). Thus, there is a strong justification for introgression of still higher level of white mold resistance recently identified in *P. coccineus* (Gilmore et al., 2002). Also, combining favorable alleles and QTL from across *P. coccineus*, Andean (e.g., A 195, MO 162, and G 122), Middle American (e.g., ICA Bunsi, 115 M), and snap bean (e.g., B 7354, CORN 501, CORN 601, and NY 6020–4) may yield yet higher levels of white mold resistance.

2.4.2.16 Bean Pod Weevil Resistance

This insect is a severe problem at medium to high elevations in Mexico and Central America. In the humid highlands of Mexico, race Jalisco cultivars are grown, whereas race Mesoamerica cultivars are popular at lower elevations in Mexico and Central America. Most of this latter group of cultivars is highly susceptible to *A. godmani*. An extremely high level of resistance (antibiosis) is found in race Jalisco (Garza et al., 1996, 2001). The *Agr* allele alone confers intermediate level of resistance, but when present

with the *Agm* allele, the resistance to the pod weevil is higher (Garza et al., 1996). However, the *Agm* allele alone has no effect. High level of resistance from the race Jalisco has been transferred into race Mesoamerica cultivars using the mass-pedigree selection method (Beebe et al., 1993).

2.4.2.17 Leafhopper Resistance

Heritable differences for ovipositional nonpreference and tolerance to feeding by leafhoppers *(E. kraemeri)* have been found only in small-seeded race Mesoamerica cultivars (Calderon and Backus, 1992; Kornegay et al., 1986). Both traits are inherited quantitatively (Galwey and Evans, 1982; Gonzales et al., 2001; Kornegay and Temple, 1986). Through recurring cycles of the bulkpedigree method of selection, significantly higher tolerance to leafhoppers was accumulated (Kornegay and Cardona, 1990; Kornegay et al., 1989). When tested in temperate environments, most of these have also shown tolerance to *E. fabae* (Schaafsma et al., 1998). However, much higher resistance to leafhoppers is found in some tepary bean (Cardona and Kornegay, 1999), such as accession G 40036, which needs to be introgressed and combined with the tolerance available in common bean.

2.4.2.18 Others

Some studies of germplasm screening, genetics, and breeding that have been carried out for bacterial brown spot; BCTV; seed coat color oxidation (Ergun et al., 2001); cooking, canning, and nutritional quality (Hosfield et al., 2000; McPhee et al., 2002; Moraghan and Grafton, 2001; Posa et al., 1999; Posa-Macalincag et al., 2002); seed coat color and pattern (Beninger and Hosfield, 1999; Beninger et al., 1999; Hosfield and Beninger, 1999; McClean et al., 2002); and seed size (Park et al., 1999, 2000) will not be discussed here.

2.4.3 Simultaneous Improvement of Multiple Traits for Cultivar Development

High-yielding cultivars with the highest expression of each trait and a combination of maximum number of desirable traits are sought in each successive breeding cycle. For each market class most rapid progress is made when all cultivars, elite breeding lines, and donors of complementary favorable alleles and QTL (including those obtained from introgression and pyramiding of favorable complementary alleles and QTL from alien germplasm) are similar in growth habit, maturity, seed color, and size—and are equally well adapted. Thus, each cross is made among only high-yielding, well-adapted, elite recipient and donor parents. When the necessary alleles and QTL for each major trait of interest are found in separate parents, bi-parental crosses and backcrosses are not adequate. A few multiple-parent crosses should be preferred over a large number of single crosses and backcrosses. Although comparatively, more time is spent during hybridization to generate multiple-parent crosses, the process allows production of recombinants with favorable alleles and QTL for multiple traits. This production of recombinants is not possible through single crosses and backcrosses without repeated

cycles of selection for specific traits, one trait at a time. Thus, all favorable alleles and QTL are combined in the first step, and the time required for cultivar development is reduced.

Gamete selection in the F_1, when combined with early generation (F_2 to F_5) selection, should help identify promising populations and families within populations (Singh, 1994). These are then used to develop superior breeding lines for subsequent cultivar identification. Use of molecular markers to select families and populations that are harvested in bulk in early generations may not be feasible currently, because of the prohibitive costs of screening a large number of plants in each generation. To recombine a recessive allele, such as resistance to leaf chlorosis induced by BGMV and BGYMV (Urrea et al., 1996; Velez et al., 1998) and complex traits that are controlled by multiple alleles, such as for seed coat color and pattern (McClean et al., 2002), intensive selection in early generations should be avoided. The frequency of desirable recombinants is very low and there is a danger of losing potentially useful recombinants that might arise in later generations. For such traits, it is preferable to initiate evaluation and selection in the F_5 onward. For abiotic and biotic stresses that cannot be screened simultaneously, different complementary locations and nurseries may be required to select promising populations and families within populations (Singh et al., 1991d, 1992a). Some progress achieved in improvement of cultivars of races Durango, Mesoamerica, and Nueva Granada, which occupy more than 80% of the area sown to dry bean worldwide, will be briefly discussed.

2.4.3.1 Race Durango Cultivars

Race Durango cultivars were domesticated in the relatively cool semi-arid central and northern highlands of Mexico. Approximately 3 million ha are planted with these cultivars worldwide. Major market classes comprise bayo (beige), great northern, 'ojo de cabra' (cream-striped), pink, pinto, and red Mexican. These have a growth habit Type III, and are mostly grown in North America. Great northern and red Mexican cultivars are also grown in Europe, North Africa, and West Asia. Early maturity, high-yield potential, high harvest index, and resistance to drought and low soil fertility are common characteristics of these cultivars. Some landraces also possess resistance to angular leaf spot, anthracnose, and resistance alleles for BCMV, BCMNV, BCTV, BGMV, BGYMV, and bean yellow mosaic virus.

Acosta-Gallegos et al. (1995b, 2001) and Singh et al. (1993) combined resistance to angular leaf spot, anthracnose, BCMV, and rust into high-yielding bayo, black, ojo de cabra, and pinto bean for Mexican highlands. Brick et al. (2001), Coyne et al. (2000), Kelly et al. (1999a, 1999d), and Myers et al. (2001a, 2001b), among others (see Beaver et al., 2003; Brick and Grafton, 1999; Singh, 2001a, 2001b), combined BCMV and rust resistance into great northern and pinto beans. Coyne (1999) lists dry bean cultivars released in the U.S. However, the most remarkable change achieved by breeding has been in the plant type, from a traditional prostrate Type III to upright Type II (Coyne et al., 2000; Grafton et al., 1999; Kelly, 2001; Kelly et al., 1999d). This milestone achievement capitalized upon germplasm and information resulting from the pioneering work of Kelly and Adams (1987).

Resistance to drought and soil compaction seems to have been inadvertently reduced in modern cultivars, compared to landraces grown until the first half of twentieth century in the western U.S. Improving resistance to these abiotic stresses simultaneously with resistance to angular leaf spot, anthracnose, bacterial brown spot, BCMV, BCMNV, BCTV, common and halo bacterial blights, root rots, rust, and, especially white mold in otherwise early maturing, high-yielding cultivars adapted to North America is the most formidable challenge.

2.4.3.2 Race Mesoamerica Cultivars

Among all common bean cultivars, these occupy by far the most hectares in the world (>6 million ha) and have the longest history of genetic improvement. However, most of the production occurs in the Americas. Most popular landraces and improved cultivars have either growth habit Type II or Type III. In Latin America, these cultivars have occupied relatively warmer climates with mean growing temperatures of 22 to 24°C. Type IV cultivars are grown in intercropping systems in reduced area in southern highlands of Mexico and Central America. Despite the fact that a few small-seeded Type I landraces (e.g., Brasil 2, Kupal) exist in Latin America, modern cultivars are mostly grown in Canada, the U.S., and Chile, where early maturity is needed. Although cultivars of more than a dozen market classes are grown, black, cream, cream-striped, navy, red, and small white are the most popular. Black bean is grown in more than 2 million ha from Canada to Argentina and Chile. The maximum variation for market classes is found in Brazil, Central America, and Mexico (see Singh, 1999c).

It is not uncommon to find landraces that possess three or more desirable attributes. For example, ‘Compuesto Chimaltenango 2’ from Guatemala is high yielding, resistant to rust, angular leaf spot, and anthracnose, and also tolerant to low soil fertility and root rots. Similarly, Ecuador 299 is resistant to rust, angular leaf spot, and anthracnose. Brazil 2 has a growth habit Type I, and is insensitive to photoperiod, and resistant to BCMV (*I* allele) and anthracnose. Carioca is high yielding, widely adapted, resistant to BCMV (*I* allele), and tolerant to BGMV, low soil fertility, drought, angular leaf spot, anthracnose, common bacterial blight, leafhoppers, and root-knot nematodes. In addition, Carioca occupies the largest area (at least 1 million ha) currently sown under any landrace or improved dry bean cultivar in the world. Another example is San Cristobal 83, which has an attractive red mottled seed color and carries the *I* allele for resistance to BCMV. This seed color and the *I* resistance allele combination is very rare in either improved cultivars or landraces because of undesirable linkage (Kyle and Dickson, 1988; Temple and Morales, 1986). San Cristobal 83 is high yielding and tolerant to drought (Singh, 1995; Terán and Singh, 2002a; White et al., 1994b).

The major achievements of breeding in North America include earliness, adaptation to higher latitude, high yield, upright plant type including a change from Type III to Type I to Type II, combination of *bc-3* and *I* alleles for resistance to BCMV and BCMNV, and rust and anthracnose resistance (Adams, 1982; Grafton et al., 1993; Kelly et al., 1994, 2000). In the tropics and subtropics of Latin America, resistance to *A. godmani,* angular leaf spot, anthracnose, BCMV, BGYMV, bruchid, common bacterial blight, and leafhopper, and upright plant type in beige, black, cream, cream-striped, and red beans

were incorporated (Beaver et al., 2003; Beebe et al., 1993; Kornegay and Cardona, 1990; Singh et al., 1998, 2000c; Thung et al., 1993).

Silva et al. (2003) in Brazil in the mid-1980s released the first cultivar, EMGOPA-Ouro-201 (synonymous with A 295), that combined angular leaf spot, anthracnose, BCMV, common bacterial blight, halo blight, powdery mildew, and rust resistance. PI 207262 and great northern cultivar Tara contributed most of these resistances to EMGOPA-Ouro-201 via A 30.

Incorporating high yield (>2,500 kg/ha) and seed quality in early maturing (<90 days from planting to harvest maturity in North America) upright cultivars suitable for mechanical harvest is a challenge. Other desirable traits include higher resistance to anthracnose, bacterial brown spot, common bacterial blight, halo blight, root rots, and white mold. Resistance to cold, drought, heat, and low soil fertility may also be required for the northern and northwestern U.S. and southern and southwestern Canada. The availability of early maturing cultivars with resistance to the above mentioned abiotic and biotic stresses would increase adoption of cultivars and facilitate low-input sustainable organic and conventional farming systems.

2.4.3.3 Race Nueva Granada Cultivars

Low to medium elevation (650 to 1850 m) of the Andes with moderately cool climate is the primary region of diversity for race Nueva Granada. Popular cultivars include light and dark red kidney, white kidney, ‘alubia’ and ‘faba’ (both cylindrical large to extremely large white), cranberry, ‘canario’ (beige), and ‘azufrado’ (sulfur) beans. Although there are some growth habit Type II, Type III, and Type IV cultivars, the Type I predominates in Africa, Asia, Europe, and the Americas. Race Nueva Granada cultivars occupy approximately 3.5 million ha worldwide.

Race Nueva Granada cultivars are poorly adapted in the highlands (above 2000 m elevation) and at higher latitudes, and most abiotic and biotic production constraints listed for races Durango and Mesoamerica also are problematic for these dry beans. These large-seeded Andean cultivars have a slower growth rate, lower dry matter yields, and their seed yields are 40 to 60% lower compared to that of indeterminate growth habit Type II and Type III cultivars of small-seeded Mesoamerica and medium-seeded Durango races (White and González, 1990; White et al., 1992).

Major advances achieved by breeding include incorporation of earliness, adaptation to higher latitude, and resistance to anthracnose, BCMV, BCMNV, BCTV, common bacterial blight, or rust in Type I cultivars of different market classes. Dark red kidney cultivar Montcalm with excellent canning quality, *I* resistance allele for BCMV, tolerance to common bacterial blight, and broad adaptation was released by Michigan State University in 1961. After 40 years it is still a popular cultivar. Good canning quality and resistance to anthracnose, BCMV, BCTV, and rust, either singly or in combinations, have also been bred into other dark red kidney, light red kidney, white kidney, and cranberry beans for North America (Kelly et al., 1998b, 1999b, 1999c; Miklas and Kelly, 2002; Miklas et al., 2002b).

Beaver (1999), Beaver and Steadman (1999), and Beaver et al. (2003) described breeding for race Nueva Granada cultivars for the tropics and subtropics. Resistance to

angular leaf spot, anthracnose, BCMV, BCMNV, BGMV, BGYMV, common bacterial blight, or rust have been bred for Africa, the Caribbean, and Latin America.

Improving seed yield of growth habit Type I cultivars, and combining high yield, high seed quality, and early maturity in growth habit Type II and Type III cultivars for North America is challenging. However, resistance to drought and low fertility soils, along with resistance to major biotic constraints will be essential for sustained common bean production in the tropics and subtropics of Africa and Latin America.

2.5 CONCLUSIONS AND PROSPECTS

Dry bean cultivation continues to be pushed into low-fertility soils and other marginal environments where drought or high or low temperature take a toll on performance. In addition, there has been a growing concern for conservation and efficient use of natural resources, including sustainable agriculture and production of organic food. To sustain families on the farm and keep up with the pace of demographic development, yield per unit of cropped land must be maximized, production costs reduced, and farming made attractive to increasingly demanding younger generations. Thus, it is essential to develop broadly adapted, high-quality, high-yielding cultivars that are less dependent on water, fertilizer, pesticide, and labor.

Significant advances have been made in germplasm collection, and a large number of accessions are available for the *Phaseolus* species in the primary, secondary, and tertiary gene pool of the common bean. Nevertheless, the genetic base of cultivars within each market class is extremely narrow. Moreover, there has been a marked reduction in genetic diversity of cultivars during domestication. Only a small portion of the available genetic diversity has been used globally thus far.

Significant advances have been made in understanding the origin, domestication, and organization of genetic diversity, germplasm characterization, and screening methods for abiotic and biotic stresses. Useful germplasm has been identified for most agronomic traits, and molecular markers for indirect selection of favorable alleles and QTL are becoming available in increasing numbers. Alternative recombination and selection methods have also been developed. While favorable alleles and QTL for a few traits have been incorporated into improved germplasm and cultivars, resistance to a maximum number of production-limiting factors has to be combined in otherwise high-quality, high-yielding cultivars with desirable plant, seed, and adaptation characteristics. This will be possible only by introgression of favorable alleles from alien germplasm, pyramiding favorable alleles and QTL (from across market classes, races, gene pools, and wild populations in the primary, secondary, and tertiary gene pool) for specific traits, and simultaneously improving the maximum number of traits for each market class of dry and snap bean. Thus, a team approach, interdisciplinary and interinstitutional collaboration, and a long-term integrated genetic improvement must be emphasized.

Transformation is beginning to be used to introgress useful alleles from *non-Phaseolus* species (Aragão et al., 2002). Molecular marker-assisted selection is being increasingly used (Kelly and Miklas, 1998; Kelly et al., 2003; Miklas et al., 2003b; Yu et al., 2000). Canning, cooking, nutritional, and processing qualities (Hosfield et al., 2000; McPhee et al., 2002; Moraghan and Grafton, 2001; Posa et al., 1999; Posa-Macalincag et al., 2002),

and genetics of seed coat color and patterns (Beninger and Hosfield, 1999; Beninger et al., 1999; Hosfield and Beninger, 1999; McClean et al., 2002) are better understood than before. Similarly, more integrated linkage maps are becoming available (Freyre et al., 1998; Gepts, 1999; Kelly et al., 2003; Tar'an et al., 2002). Researchers should therefore strive to combine the best of conventional and modern molecular approaches to improve common bean germplasm and cultivars for the benefit of humankind.

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CHAPTER 3
Pea (Pisum sativum L.)

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3.1 INTRODUCTION

New research in pea (*Pisum sativum* L.) has the potential to contribute exciting new dimensions to the conventional exploitation of genetic resources and breeding strategies for improved varieties. This chapter examines a range of topics, including conventional studies of cytology, taxonomy, genetic resources, plant breeding with manual crossing, and nitrogen fixation; and new techniques in interspecific gene transfer and the use of molecular markers, which both widen the scope and improve the precision of conventional approaches.

The first topic in section 3.2 overviews crop description and use, section 3.3 outlines current knowledge of the pea germplasm and its wild relatives and leads into section 3.4, which gives an overview of objectives being pursued in terms of conventional breeding. Legumes have a special place in agricultural systems; their contribution to both crop nutrition and soil fertility has co-evolved with rhizobia in the symbiotic fixation of soil atmospheric nitrogen. This topic, covered in section 3.5, follows conventional breeding, since parallel progress in biological nitrogen fixation is an important consideration for realizing the benefits of using pea as grain, fodder, and vegetable crops.

Section 3.6 gives an overview of molecular variation in the species. Molecular techniques for analyzing the pea genome are outlined, as well as their application to investigating intraspecific variation, mapping the pea genome and their potential for more targeted and precise breeding through marker-assisted selection.

The final broad topic (section 3.7) on plant tissue culture provides a new source of genetic variation, as well as enabling tools for interspecific transformation. The latter holds the promise for crop benefits through tapping genetic resources from the worldwide flora, with important reductions in the use of agricultural chemicals and new capacities for pest and disease resistances. In responsive species, tissue culture also enables double haploid production, which provides options for accelerated breeding and a tool for the development of homozygous populations for molecular mapping applications. A final summary (section 3.8) indicates the many pathways open for crop improvement in the future.

3.2 DESCRIPTION AND CROP USE

3.2.1 Botany and Inheritance of Morphological Traits

Cultivated pea (*Pisum sativum* L.), 2n=14, is in the genus *Pisum* of family Fabaceae, synonymous with family Leguminosae. Cultivation of pea includes diverse types and systems, widely dispersed around the world in temperate to elevated subtropical environments.

The seed mainly consists of two cotyledons, which provide initial growth support to the embryo, with a primordial root or radicle, stem tissue, and an embryonic growing point, or plumule. Germination is epigeal. The seed has a hilum, with which it was attached to the maternal pod tissue during development, and near this is a micropyle, where the germinating radicle emerges (Makasheva, 1983). The taproot has many lateral branches and sub-branches, which are colonized by symbiotic nitrogen fixing *Rhizobium leguminosarum* bv. *Phaseoli* to form nodules.

A major gene (Le/le) for internode length is mainly responsible for tall climbing (Le/-) vs. dwarf bush (le/le) habit (Hebblethwaite et al., 1985). Height ranges from less than 50 cm (dwarf to more than 300 cm tall). Stems are usually weak and prone to lodge if unsupported. A stipule and leaf stalk are attached to the stem at each node. Nonbearing (sterile) nodes occur on the main stem from the lowest node upward, with frequency correlated with time to flowering. This trait is genetically defined for specific environments and reflects the length of the vegetative period before the appearance of the reproductive fruiting nodes (Makasheva, 1983).

Major genes control expressions of terminal tendrils, tendrils without leaflets or terminal minute unpaired leaves, leaf shape, dentation of leaf margins, stipule size and shape; and the color, size, and shape of flowers, pods, and seed. Flower number per node, seeds per pod, and seed size (10 to 36 g/100 seed) are affected by both major and quantitative genes. Peas are long-day responsive for timing of reproduction, with up to six major genes controlling a range of flowering behavior from day neutral, early initiation, late, late high photoperiod response to very late (Hebblethwaite et al., 1985).

Major genes for reproductive traits include corolla color of flowers—white, pink, or purple carried on racemes. Pod traits range from wide flattened to round and dehiscent on both sutures, with immature color from yellow to dark green. Pigmented patterns sometimes present with mature color, ranging from light yellow, brown to violet brown. Seed shape ranges from spherical, angular to partially compressed, with smooth to wrinkled surfaces (Duke, 1981).

3.2.2 World Production and Utilization

In 2001, world production of dry peas was 10.3 million tons (USDA). Major producers were Canada (21.1%), France (15.5%), China (11.3%), and Russia (9.7%). Up to 25% of pea crops are consumed as fresh green seeds and pods—and as foliage, which may be directly eaten, home cooked, or processed as canned or frozen produce. India produced 3.1 million tonnes (wet weight) in 2001–2002, the U.S. produced 460,000 tons of green pea for processing in 1997, and China had 160,000 ha of vegetable pea production in 1999 (Redden, personal communication, 2001). Peas are highly nutritious with a grain

protein of 26%, range 16 to 40% (Newman et al., 1988; Bressani and Elias, 1988). Pea is grown for fodder or for green manure in Eastern Europe and Canada (Makasheva, 1983).

3.3 TAXONOMY AND GERMPLASM RESOURCES

3.3.1 Centers of Diversity and Taxonomy of Pea

The earliest date for the appearance of domesticated pea is 7000 BC from Turkey to Iran, and it was grown 1000 to 2000 years later in Greece and Europe (Ambrose, 1995; van der Maesen et al., 1998). The centers of diversity associated with the domestication of pea are the fertile crescent (Turkey, Syria, Iraq, and Lebanon), the Mediterranean, and central Asia—with Ethiopia possibly a secondary center of diversity (van der Maesen et al., 1998). Zohary (1999) implied that pea domestication events were infrequent. The wild progenitor of pea is uncertain, although Ben-Ze'ev and Zohary (1973) favor *P. sativum* subsp. *humile* found in steppe habitats and crop verges. Changes with domestication included the development of larger seed, loss of pod dehiscence, and a shorter compact habit (Davies, 1976).

Pea is classified by the United States Department of Agriculture (USDA, 1990) as *Pisum sativum* ssp. *sativum* L. in the genus *Pisum*, which contains only one other species, the wild *P. fulvum* Sibth. & Sm. from the eastern Mediterranean, but taxonomic classification in *Pisum* has diverged (Duke, 1981; Wiersema et al., 1990).

Pisum fulvum has: unique isoenzyme alleles (IBPGR, 1988); a short habit with basal branching of thin stems; small flowers with corolla light yellow to dark orange; dehiscent pods; and small, round seeds, dark brown in color, with a thick, finely granulated seed coat. It is found wild among rocks from Turkey to Arabia (Makasheva, 1983). F_1 hybrids between *P. sativum* and *P. fulvum* are abnormal with irregular meiotic pairing, and only succeed when *P. fulvum* is the pollen parent, indicating both cytological and chromosomal differences (Ben-Ze'ev and Zohary, 1973).

Many subspecies are recognized within *P. sativum*, with differences in major gene traits and in distribution as distinct ecotypes (Ambrose, 1995), which in the past have been classified as separate species or synonyms (Zohary, 1999). All pea subspecies are fully cross-fertile, whereas subspecies *P. abyssinicum*, cultivated in Ethiopia and Yemen—and also wild in Ethiopia—may have evolved independently with contributions of *P. fulvum* and *P. elatius* germplasm (Makasheva, 1983). Synonyms of *P. abyssinicum* are *P. abyssinicum* var. *vacilaianum* and *P. jombardii* (USDA, 1990). *Pisum abyssinicum* has a waxy coat on the stem, giving a bluish tinge plus extensive pigmentation on juvenile stems and leaves; colored flowers; and glossy seeds that are irregularly rounded and greenish grey to dark violet (Makasheva, 1983). A range of pea species and subspecies are shown in Figures 3.1–3.5.

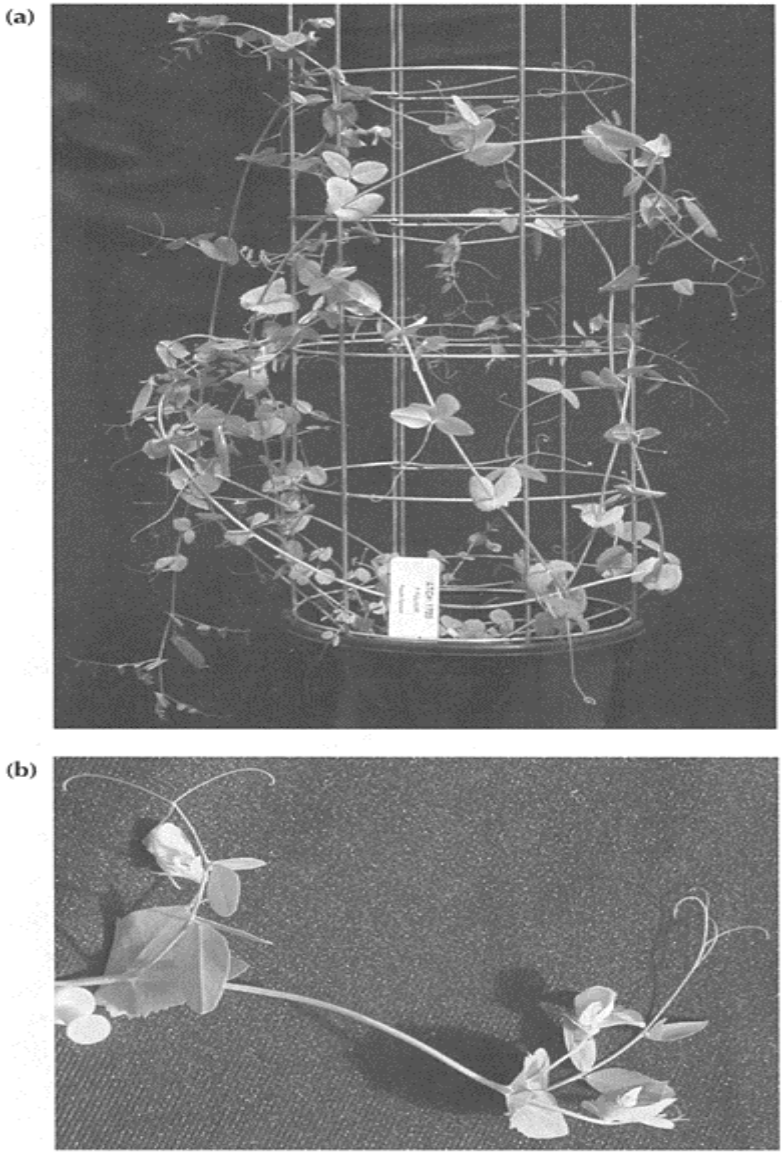
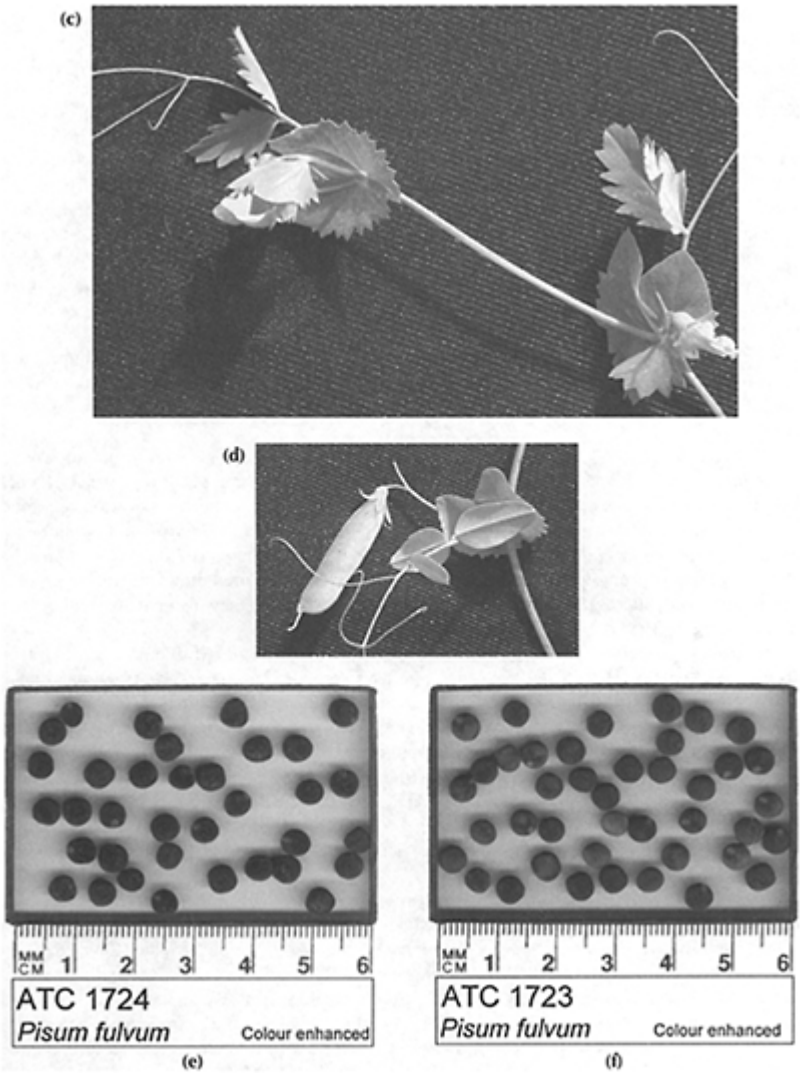


Figure 3.1 *P. fulvum* (a) plant—ATC 1723; (b) flower—ATC 1723; (c) serrated branch—ATC 1724; (d) pod—ATC 1723; (e) *Pisum fulvum* seed—ATC 1724; (f) seed—ATC 1723.



Subspecies *P. elatius* [(Steven and M. Bieb) Asch. & Graebn.] grows wild in humid forested mountain valleys from the Caucasus to Mediterranean regions and can occur as a weed in wheat fields in Georgia (Makasheva, 1983). *Pisum elatius* has a chromosomal translocation difference from *P. sativum* with intergradations found between domestic pea and *elatius*, and morphological boundaries separate *P. elatius* and *P. humile* (Ben-Ze'ev and Zohary, 1973). *Pisum elatius* has dehiscent pods 6 to 8 cm in length, finely granulated seed coats colored grey with varying spotted to marbled violet patterns, and stiff stems toward maturity. Stipules may also have an anthocyanin ring (Makasheva, 1983).

Subspecies *P. pumilio* [(also *P. sativum* L. subsp. *elatius* (Steven and M.Bieb) Asch. & Graebn. var. *pumilio* Meilke)] is a fodder type with shorter internodes, peduncles, and

pods and small flowers. *P. pumilio* is distributed from Greece to Iran in steppe-type habitats as a weed on crop verges and can undergo spontaneous hybridization with cultivated pea. It includes subsp. synonyms: *humile*—semicultivated and wild with subpopulations from Israel differing by a chromosomal translocation from *P. sativum*, and *syriacum*—short statured with small pods, also found wild in southern Asia to the Middle East (Ben-Ze'ev and Zohary, 1973; Makesheva, 1983). Some taxonomists place *P. humile* as a variant of *P. elatius* (Ambrose, 1995), whereas *P. humile* from its northern distribution has the same karyotype as domestic pea and may be its progenitor, possibly in association with *P. elatius.*

Subspecies *P. sativum* var *sativum* has subsp. synonyms *commune, hortense,* and *speciosum*. The latter has pigmented flowers and seeds, which turn brown in storage. Subspecies *P. arvense* are the fodder pea types, and subspecies *P. sativum* var. *macrocarpon* Ser. are the sugar, snap, and snow peas with edible pods (Gaskell, 1997). Subspecies *P. transcaucasicum* Govor., distributed in southern Russia, has thin stems, long internodes, acute tapering stipules, small angular seeds with brown and marbled seed color often with violet mottling. Subspecies *P. asiaticum* is a primitive form of garden pea cultivated in central and western Asia, with thin stems and basal branching (Makasheva, 1983). *Pisum formosum* has been reclassified as *Vavilovia formosa* (Steven) Fed. It is an ornamental type, with carmine flowers, found in southwestern Asia (Duke, 1981).

Evolution through mutation continued post-domestication, including garden peas selected for sweeter wrinkled as well as smooth seed, snow peas selected for thin-walled pods that lack fiber, and snap peas selected for thickened pod walls also low in fiber (Myers et al., 2001).

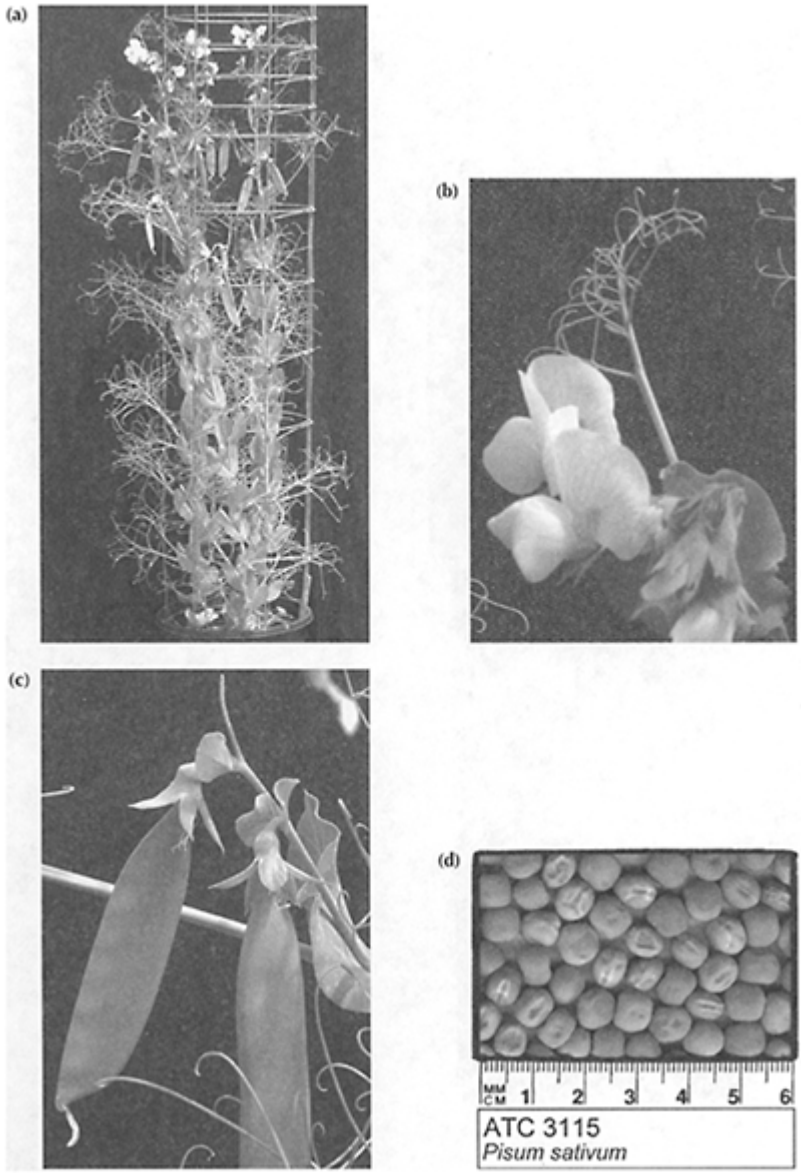


Figure 3.2 *Pisum sativum* (a) plant—ATC 3115; (b) flower—ATC 3115; (c) pods—ATC 3115; (d) seed ATC 3115.

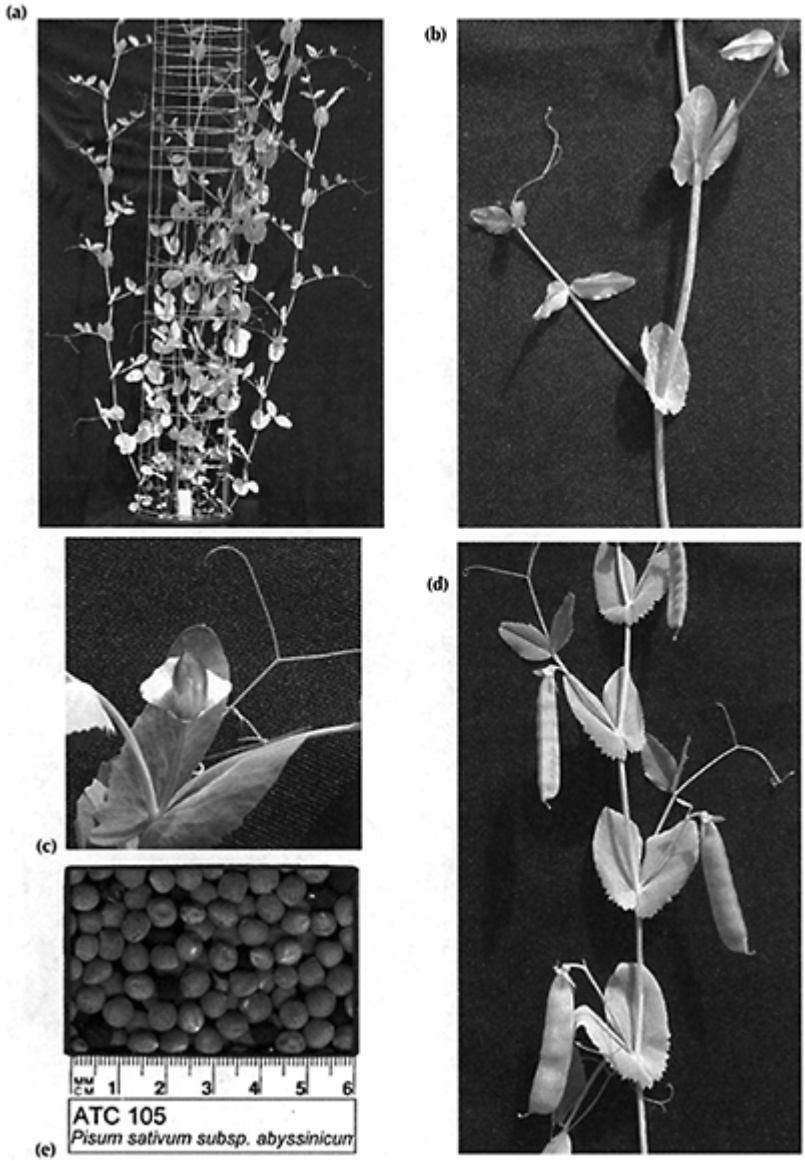


Figure 3.3 *Pisum sativum* subsp. *abyssinicum* (a) plant—ATC 105; (b) branch—ATC 105; (c) flower—ATC 105; (d) four pods—ATC 105; (e) seed—ATC 105.

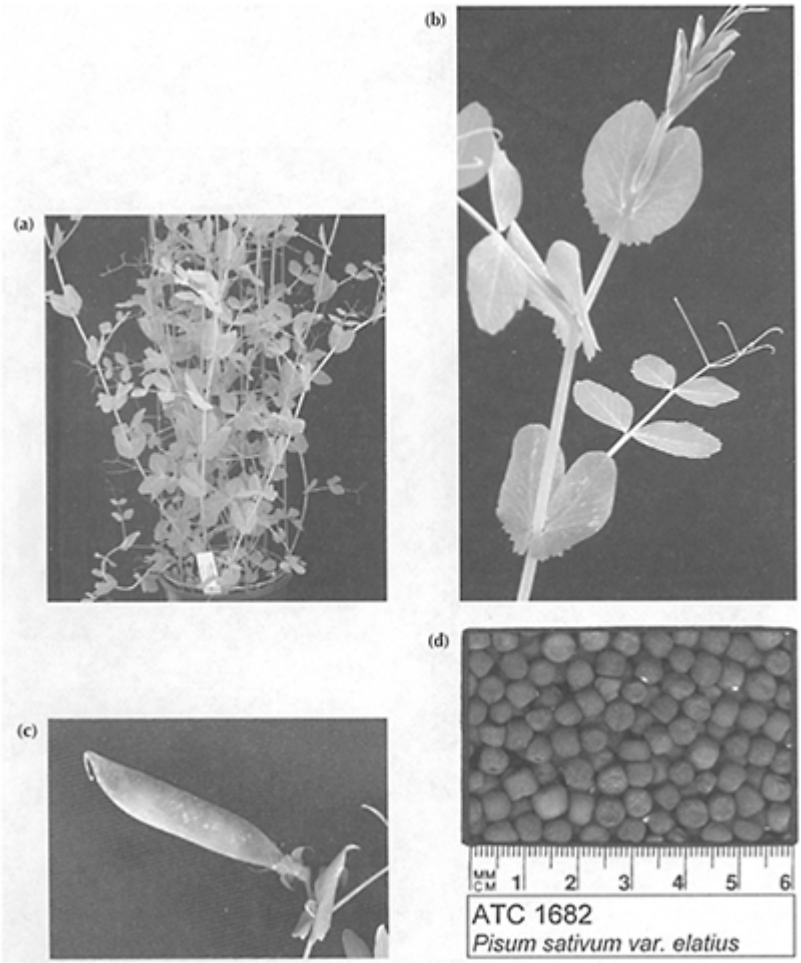


Figure 3.4 *Pisum sativum* var. *elatius* (a) plant—ATC 1682; (b) branch—ATC 1682; (c) pod—ATC 3340; (d) seed—ATC 1682.

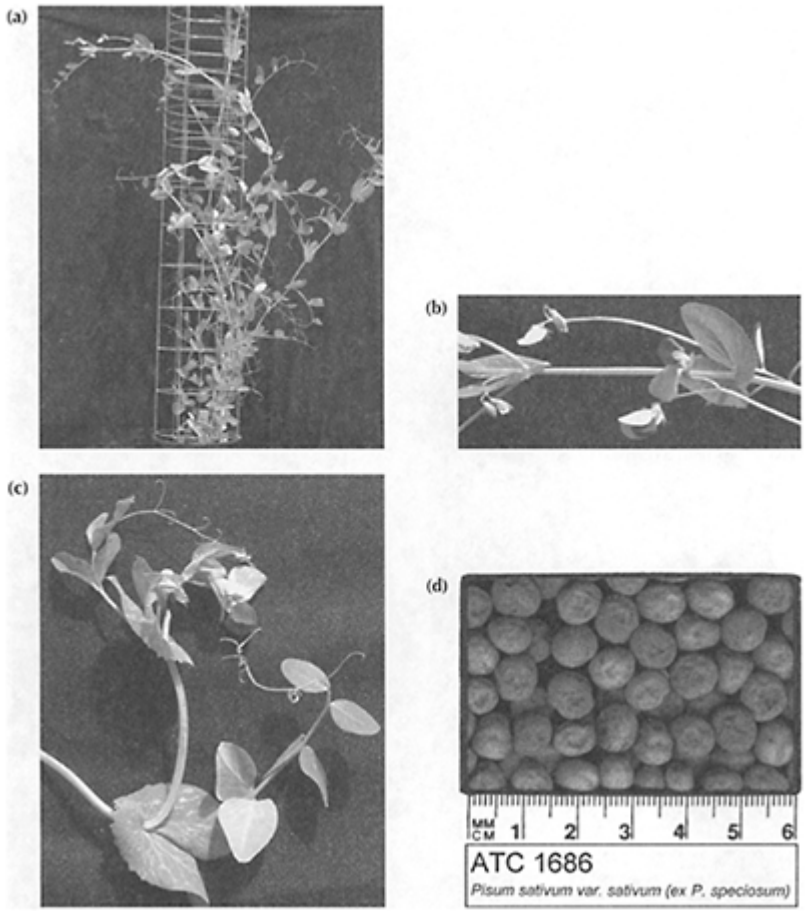


Figure 3.5 *Pisum sativum* var. *sativum* (ex *P. speciosum*) (a) plant—ATC 1695; (b) branch—ATC 1695; (c) two flowers—ATC 1686; (d) seed—ATC 1686.

Grain and vegetable types have a white flower corolla with white seed color. Yellow to pink, though green seed is also documented in Russian cultivars (Makasheva, 1983). Most American and European cultivars have white flowers. Genetic diversity is greater among colored flower types, and there is a higher frequency of rare allozymes among Turkish accessions (IBPGR, 1988)—consistent with Turkey being in a center of diversity. Makasheva (1983) lists germplasm from a range of Middle Eastern and Southern Asian locations, with corresponding synonyms and regional variants for morphological, physiological, abiotic stress tolerance, and disease resistance traits.

Table 3.1 *Pisum* sp. Genetic Resource Collections

Country	Genebank	Accessions
ICARDA Syria	ICARDA germplasm unit, Aleppo	5935
USA	USDA, Washington D.C.	4384
Italy	Institut del Germplasma, Bari	4558
Russia	N.I.Vavilov All Russian Science Research Institute of Plant Industry, St. Petersburg	6790
Sweden	Nordic Gene Bank, Alnarp	3921
United Kingdom	John Innes Institute, Norwich	3030
Germany	Zentral Institut Genetik Kulterplanzen, Braunschweig/Institute of Plant Genetic Resources and Crop Plant, (PGRDEU), Research, Gaterslaben	3184
Poland	Plant Breeding Institute, Wiatrowo	2899
China	Institute Crop Germplasm Research (ICGR), Beijing	2292
Czech Republic	The Research Institute of Crop Production, Department of Gene Bank, Olomouc	2364
Australia	Australian Temperate Field Crops Collection (ATFCC), Horsham	2283
Hungary	Institut for Agrobotany, Tapioszde	2282
France	Laboratoire des Legumineuses, URGAP-INRA, Versailles	1850
Bulgaria	Institut for Introduction and Plant Genetic Resources “k.Malkovo,” Plodiv	1490
Slovakia	Genebank of Slovak Republic, Piestany	1105
Netherlands	Centre Genetic Resources, Wageningen	1008

3.3.2 Germplasm Resources

3.3.2.1 Ex situ *Collections*

Genetic resources of pea include both the traditional cultivars and wild relatives and still occur as *in situ* collections in centers of diversity. Genetic resource centers worldwide maintain *ex situ* collections of seed (Ambrose, 1995). To maintain seed viability, seed

moisture is usually lowered by preliminary storage in a dehumidified atmosphere. Then seed is stored in "active" collections at 1 to 5°C for distribution of seeds to breeders and researchers utilizing germplasm to source desirable traits, and at −18 to 20°C in "base" collections for long-term preservation for future agricultural needs. There may be a "backup" collection at another location, also stored at low temperature. Seed in the active collections is regularly regenerated, commonly at a 10-year interval or when viability decline is observed to fall in monitored germination tests. Collections are widely exchanged between genetic resource centers, and the respective identifier codes and names are added to the passport data as synonyms.

Collections ideally have passport information on the geographic and agri-ecological descriptions of locations where traditional varieties (landraces) and wild relatives are collected. Collections also might have additional data on the evaluation of collections for agricultural production and biotic and abiotic stress traits, which characterize genetic diversity and assist in its strategic utilization for crop improvement (Maxted et al., 2000).

The success of modern plant breeding in producing input responsive varieties led to displacement of traditional varieties in the major centers of diversity, raising concerns about the ensuing loss of genetic diversity. Landraces have genetic combinations resulting from evolutionary adaptation to local climatic and soil variability plus accumulated mutations, and manual selection for either crop husbandry (e.g., height) or for aesthetic and end-use products (e.g., seed size, color, and cooking quality). They contain unique and rich genetic diversity, which is lost when farmers adopt modern varieties with a much narrower genetic base (Frankel and Bennett, 1970). Future progress in plant breeding depends on the continued availability of this diversity for current and future challenges with environmental and sustainability concerns (Muehlbauer and Tullu, 1998). With the erosion of *in situ* collections in centers of diversity, pea improvement is now dependent on *ex situ* collections.

Table 3.1 lists collections with more than 1000 pea accessions in 2002–2003 on the Internet and in other publications (IBPGR, 1990). The International Centre for Agricultural Research in the Dry Areas (ICARDA) collection has been extensively distributed, and much of the Vavilov collection has been received at the Australian Temperate Field Crops Collection (ATFCC). The Vavilov Centre extensively collected from the 1920s, and has the most representative collections from centers of diversity where significant genetic erosion has occurred (FAO IBP, 1973).

Although the aggregate of these pea collections exceeds 49,000, it is small in comparison with the cereal collections, which includes more than 410,000 wheat accessions and more than 210,000 rice accessions in *ex situ* collections worldwide (Tanksley and McCouch, 1997). Further collection of remaining landraces—and of wild relatives—is an urgent issue, given the loss of habitat and displacement by modern varieties (Ladizinsky, 1998). Centers of diversity are rich in genetic diversity for tolerance to a wide range of soil, mineral, and climatic stresses and for resistances to significant mildew, wilt, and viral diseases (Duke, 1981). Proposed conservation approaches include missions for rescuing *in situ* collections, filling geographic gaps, and sampling specific habitats where "hot spots" for specific traits have been identified (Ladizinsky, 1998).

3.3.2.2 *Wild and Weedy Relatives*

There is a well recognized "founder" effect associated with domestication of favorable mutations in pea for nondehiscence: more and larger seed and less dormancy, which resulted in a very limited sampling of the genetic diversity available in wild relatives (Zohary, 1999).

Ex situ collections of wild and weedy relatives of pea are poorly represented in collections. Accession numbers of *P. fulvum* are 36 with the U.S.Department of Agriculture (USDA), 13 with the central documentation site for *ex situ* collections of plant genetic resources in Germany (PGRDEU), and 51 with Australian Temperate Field Crops Collection (ATFCC). *Pisum elatius* and *P. sativum* var. *pumilio* synonym *humile* have 43 and 10 accessions, respectively, with the USDA, 14 and 3, respectively, with ATFCC, and PGRDEU has 23 *P. elatius* accessions. Wild relatives of pea are potentially significant for genetic diversity in various adaptation traits, and higher levels of disease resistances than the cultivated pea germplasm (Maxted et al., 2000). Resistance to pea weevil, *Bruchus pisorum* (L.) has been identified in accessions of *P. fulvum* and is being transferred to *P. sativum* (Byrne et al., 2002). *Pisum fulvum* appears to be both more drought and heat tolerant with a more xeric distribution of native habitats in Israel than *P. elatius* or *P. humile* (Ladizinsky and Smartt, 2000). Further collection of wild and weedy pea species is a high priority for their centers of origin, where they tend to be scattered in isolated diverse environments (van der Maesen et al., 1998).

Quantitative trait loci (QTL) from wild relatives can also be utilized in plant breeding. By using advanced backcrosses and molecular linkage maps, subsets of "wild" alleles can be examined in the genetic backgrounds of elite cultivars and introgressions that confer superior performance can be identified, as is the case for tomato and rice (Tanksley and McCouch, 1997). These approaches could be applied to the exploitation of wild relatives of pea.

3.3.2.3 *Core Collections*

The challenge is to raise utilization of collections by breeders above the current 1 to 5% level (Goodman, 1990). *Ex situ* collections are often far too large for direct searching of desired genetic expressions. Frankel (1984) proposed the formation of core collections, which are representative samples of the whole collection. A core comprising 10% of the whole collection should retain more than 70% of the alleles in the original collection, and can be composed to represent the major genetic and ecological categories in the collection (Brown and Spillane, 1999).

This reduction of collections to a manageable size would allow systematic evaluation of genetic diversity for agriculturally important traits and the capacity to search for new genetic variation for emergent challenges, such as new disease strains or new insect pests. This approach would encourage greater use of collections by breeders and by molecular biologists, with novel tools for efficient DNA screening of germplasm and for studies of germplasm diversity and crop evolution (Brown and Spillane, 1999). Core collections are a point of entry to the entire collection rather than a separate entity (Maxted, 2000).

Pea core collections exist in the USDA, John Innes, and ICARDA centers. Core collections for pea will be developed at both ATFCC Australia and at the Institute for Crop Germplasm Research (ICGR), China, as part of a collaborative project. This will include evaluation of both collections in both countries for biotic and abiotic stresses and agronomic traits, plus Amplified Fragment Length Polymorphism (AFLP) characterization of diversity in both the core collections and wild and weedy relatives (Redden, personal communication, 2003). Core collections can usefully include wild relatives, both *P. fulvum* and the *humile, pumilio,* and *elatius* subspecies of *P. sativum.*

Core collections can assist in identifying agri-geographic clusters that are prospective hot spots for particular traits. Such geographic sources are not always predictable. Ecuadoran faba bean germplasm was found to contain high levels of resistance to chocolate spot *(Botrytis fabae),* even though this location is a relatively recent secondary source of diversity (Marcellos et el., 1999). With specific latitude and longitude data for accessions, and with super-imposition of climate and soil data, germplasm sources may be targeted for specific traits associated with natural selection in prospective localities.

3.3.2.4 Special Genetic Stocks

Pea is very rich with a large number of major gene mutations, which has made it an attractive species for genetic studies of linkage, pleiotropic, and allelic expressions. Mendel (1865) used clearly differentiated morphological mutants to establish the principles of allelic inheritance, segregation, and dominance. These included; round (R) vs. wrinkled (r) cotyledons, yellow (I) vs. green (I) cotyledons, colored (A) vs. white flowers, parchmented (V) vs. parchmentless (v) pod wall, green (Gp) vs. yellow (gp) color of unripe pods, nonfasciated (Fa) vs. fasciated (fa) apical flower position, and long (Le) vs. short (le) internode. A very large number of mutants and major genes in pea are known for physiological, chlorophyll, seed, root, shoot, foliage, inflorescence and flower, and pod traits (Blixt, 1972). These can strongly affect plant morphology, habit, adaptation, and disease resistance expressions.

A collection of special genetic stocks with well-identified phenotypic markers of high penetrance are maintained and distributed by the John Innes Centre (Ambrose, 2000), with “base” and “backup” collections in the Nordic gene bank (Blixt and Williams, 1982). Blixt (1972) classifies morphologic and physiologic mutants for a seven-chromosome linkage map. Multiple alleles occur at particular loci, including intragenic recombination, and loci are described that are relevant to domestication, disease resistance, and plant breeding for fodder, vegetable, and grain types of pea (Swiecicki et al., 2001).

Murfet and Reid (1993) describe developmental mutants that modify the overall structure or architecture of pea. Studies of pea genetics have provided a model system for studies of allelic modification of gene expressions, fine interval mapping of genes to chromosomes, and integration with DNA markers (Swiecicki et al., 2001).

3.3.2.5 Germplasm Evaluation and Use

The John Innes collections are comprehensively documented for well-expressed traits of direct crop value, including seed size and color and color patterns, cotyledon color, and

the afila mutation; the latter modifies compound leaves to tendrils and reduces lodging and susceptibility to *botrytis* and *sclerotinia* (Hawtin et al., 1998).

The USDA has the most extensively evaluated collection, with evaluation data for up to 54 traits observed. These range over seed assays for 11 different minerals; reactions to 5 diseases and race pathogens; 2 nematode species; 25 morphological traits, including seed and pod color, pigmentation patterns, size, and shape; 5 measures of phenology; and 6 growth and production traits, including grain yield. These traits are more extensively recorded for flowering date and seed descriptors than for mineral content. This extensive evaluation database adds value to the collection, for selection of accessions with desired trait combinations to use as parents in breeding programs or for specific research projects. Eventual worldwide coordination in collation of evaluation data could have the potential for improved efficiency in exploitation of germplasm by breeders.

3.4 GERMPLASM ENHANCEMENT—CONVENTIONAL BREEDING APPROACH TO IMPROVE PEAS

3.4.1 Introduction

Peas are an important crop in many arable regions of the world. They are widely used for human consumption, stockfeed, and forage purposes. They are also important within dry-land cropping systems both as a cash crop and for the provision of early soil nitrogen from nitrogen fixation.

Peas are diploid (2n=2x=14) and highly self-pollinating (99%) (Gritton, 1980). Breeding strategies adopted have, therefore, been similar to other self-pollinated crop species and generally have involved hybridization among cultivars or between cultivars, landraces, and primitive forms, followed by combinations of pedigree, bulk, backcross, or single-seed descent methods of selection. No method of producing haploids has yet been developed to fast track the selection of homozygote lines from segregating populations, but such innovation would greatly facilitate breeding.

Breeders have at their disposal a large amount of genetic diversity from both the primitive and cultivated forms of peas that can be easily intercrossed, with the exception of *P. fulvum.* Even though many wild forms show chromosomal changes that can cause partial sterility of hybrids produced, gene exchange is still possible and useful to breeders.

Peas have a very long history in cultivation and, hence, targeted selection of types or populations better suited to local farming systems and end-purpose requirements have occurred over hundreds of years. The transition from wild type to domesticated type has involved gradual changes in a number of major attributes mostly under simple genetic control. Over the last 50 years, breeders have made dramatic ideotype changes to better adapt the crop to broad-acre mechanized systems of farming.

3.4.2 Breeding for End Use

3.4.2.1 Grain

The pea grain is very versatile in its end use and very important as a source of protein in both human diets within developing countries and for stockfeed industries. In addition, components of the grain, such as starch and fiber, are increasingly used as additives in food ingredients in many developed countries. Peas can be classified into two main groups: those harvested at the green or immature stage of development (vegetable-type peas) and those harvested at the physiologically mature stage (field or dry peas).

Peas that are harvested at the immature stage can be subdivided into either (a) garden or vining types, which are shelled, and the green pea is then used to produce canned or quick-frozen products or (b) edible podded types (Chinese, snow, snap peas). Peas that are harvested dry have a wide range of market subdivisions, based mostly on physical appearance of the grain but also on suitability to processing. Whole grain peas are used in markets for canning, production of snack food types, stockfeed (various), and as an ingredient in cooking (e.g., soups). Split peas are produced and used for soups (yellow and green split peas) and *dhal* (yellow split peas).

Derived products from the field pea grain are also used as additives in food, such as in bakery products, to improve crunchiness and viscosity (e.g., starch); meat binding (e.g., fiber); emulsifers in sauces (e.g., protein); and in nondairy products (e.g., protein), but they have not yet been directly targeted by breeding.

Breeding for specific grain and pod quality types has mostly focused on selection of major genes that control seed starch content and physical appearance of the seed and pod. For example, the major gene, R, has the most profound effect on seed form and is basically responsible for the division into field pea (R) and vegetable pea (r), principally by affecting the starch-sugar content, which also visibly affects their form when ripe. The more starchy peas are smooth and almost spherical, while the less starchy vegetable peas are wrinkled when dry. Kooistra (1962) classified three types of seeds based on the association of two major genes:

R rb (wrinkled seeds with simple starch grains)
r Rb and r rb (wrinkled seed with compound starch grains)
R Rb (smooth seeds and simple starch grains)

Breeders have also utilized—or tried to reduce the effect of—other major genes that cause impressions over grain testa and radicle *(fov, sul, di, foe, l, mifo, pla)* or alter the shape from spherical to cubic *(com, qua)* (Blixt, 1977). Selection for seed size has been driven by the market requirements and has ranged from small (e.g., bird seed types) to very large (e.g., marrowfat types). Seed size appears to be quantitatively inherited; however, three major genes can cause a marked effect *(par, sg-1, sg-2)* on size (Blixt, 1977).

Seed color and patterns are very important, particularly for the dry pea market. Most major markets prefer a uniform color with no visible markings. Up to 48 genes interact to determine seed color and pattern (Blixt et al., 1978). Testa colors range from translucent,

which incorporates the major grain types marketed (blue and white peas) to ochre *(och)*, salmon *(sal)*, red brown *(oh)*, umbra *(umb)*, and violet *(U)*. Testa patterns include marbling *(M)* (i.e., maple type peas used in bird seed stockfeed), violet spots *(F, Fa)*, forks or moons of discolored areas *(dem, den, cal, cat, mp, ve, z)* brown *(Rf)*, ochre *(gl)* and grey colors *(Ca, rag)* over the radicle, and brown *(str)* and grey *(gri)* strips. The major gene for anthocyanin formation, A, is recessive and epistatic to the majority of these genes (Blixt, 1972).

Colored-type peas are widely produced and in the past were classified into various subspecies. Colored peas are also widely used for local subsistence farming in Asia and Africa. Their international trade is minor, mostly as dun type peas from Australia, forage winter types (Austrian) in North America, and maple types produced mostly for the small bird seed trade. Australian duntype peas are the most significantly traded colored type peas and are preferred for the yellow pea market in the Indian subcontinent region because of taste. To improve the splitting efficiency of dun types, breeders in Australia have reduced associated dimples by selecting for smoother seed coat types (e.g., variety Kaspa).

An important major gene (i) affects the color of the cotyledons. Most peas traded internationally are yellow split types (I) while green split and vegetable types (mostly) are recessive for the gene *i*. For green dry peas in particular, the intensity of the color is very important as a quality criteria. Breeders have been able to select for increased bleaching resistance mostly caused by exposure to sunlight prior to harvest or following prolonged storage.

3.4.2.2 Pod

The critical trait that separates edible podded peas from field peas or garden peas is the lack of parchment in the pod, which is controlled by two major recessive genes, *p* and *v*. Within the edible podded-type pea group there are two types: the thin pod wall type (snow pea types) that lack fiber in the pod wall, and the thick pod wall type (sugar snap peas), where the pod wall develops tightly around the seed and becomes round in cross section at maturity.

Breeders have been able to select for thicker and more succulent podded types by selecting for the major gene, n, which increases pod wall thickness in combination with reduced fiber. Snow pea types have also developed types with and without anthocyanin, which can markedly vary the taste.

3.4.2.3 Dry Matter Production

Plant types that have high basal branching (Austrian winter) or produce high amounts of dry matter prior to flowering (e.g., variety Morgan) provide excellent opportunities for green manuring or forage and reduce the risk of total crop loss if seasonal conditions prove unfavorable for grain production.

3.4.3 Breeding for Adaptation

3.4.3.1 Plant Type

Breeders have been able to manipulate the plant ideotype to better adapt the crop to mechanized harvesting. The older style of pea could be described as tall, leafy, and with a trailing scrambling habit at maturity. This plant ideotype is still widely grown (e.g., Australia), but requires specialized harvesting equipment to pick the crop up when dry and can be particularly susceptible to lodging and crop losses if rain occurs at maturity.

A major breakthrough came about when breeders combined reduced crop height (e.g., *le*) and conversion of leaflets to tendrils (e.g., *af*), described as the semidwarf, semileafless ideotype (Snoad, 1985). The semidwarf, semileafless ideotype provided a number of benefits, such as reduced leafiness and excessive overshading, increased aeration and reduced disease spread in some environments, and improved ease of harvest of both garden and field pea types as consequence of reduced lodging.

For the dry pea crop, breeders have selected types that are significantly more lodging resistant at maturity in local environments. The genetics that control lodging resistance appear complex. However, significant progress has been made by breeders by selecting for reduced lodging and good sward height over seasons and regions, mostly in combination with reduced internode length and in many cases with increased tendrils. A consequent negative effect of selecting for lodgingresistant types at maturity has been an increased propensity for pod shattering, particularly in environments where the end of the season is hot and dry (e.g., Australia) (Leonforte, personal communication, 2003). To overcome pod shattering, breeders have increasingly incorporated reduced pod parchment genes *(p v)* to eliminate pod dehiscence in stiffer-stemmed backgrounds.

3.4.3.2 Phenology

The length of the growing season, associated abiotic constraints at podding, and grain quality issues greatly influence the phenology targets for regional variety development. Breeders have manipulated the time to flowering, flowering duration, and rate of pod development individually in different plant backgrounds to improve crop adaptation and grain quality. While the genetics controlling flowering and pod development appear complex, several major genes have been identified, which affect flowering time *(Lf)* and photoperiod response *(Sn, Dne, Ppd, E, Hr)*. Various mutant types *(gi, fsd, Veg1, Veg2, dm, det, and fds)* have also been identified, and their interaction described (Murfet and Reid, 1993).

For processing peas, uniform maturity and concentrated pod set are necessary to maximize both yield and quality during a once-over mechanized harvest. For field peas, uniformity of grain physical features can also be improved by reducing flowering and podding time. Long flowering duration can be an advantage in environments prone to hostile stress during flowering but a disadvantage in very short-season environments where the harvest window is very short.

3.4.4 Grain Yield Potential and Stability

Yield *per se* remains a challenge. The average yield of dry peas can be more than 5 tonnes per hectare in regions of Western Europe and North America, but globally, it is seasonally between 1.5 and 2.0 tonnes per hectare, on average. The harvest index of peas in Europe and North America, where mostly dwarf varieties are grown, is comparable with modern varieties of wheat (Davies, 1985). However, in more hostile zones (e.g., Australia), where breeders have had to maintain higher biomass for stability of yield, there is still opportunity to improve the crop's harvest index even in the more dwarf genotypes.

A major challenge for breeders is to increase the yield potential of peas to that equivalent to wheat in many parts of the world. An obvious barrier is the higher protein content of peas, which requires substantially higher energy requirement per unit gain weight than starch. However, even when we take this into consideration, the yield potential of peas in some regions, as a dry crop, is substantially lower than the potential (E) and, to some extent, reflects less breeding effort and the consequent lower tolerance and resistance to biotic (e.g., disease) and abiotic constraints.

3.4.5 Improved Resistance to the Biotic Constraints

3.4.5.1 Fungal Diseases

3.4.5.1.1 Fungal Root Diseases

Fungal seed and seedling diseases such as *Pythium* and *Rhizoctonia* (seed and seedling rot) are of relatively minor importance and are mostly controlled with seed fungicide treatments and cultural practices. However, resistance to epicotyl rot caused by rhizoctonia can be improved by selecting for thicker seedling epicotyl.

Aphanomyces root rot is one of the most widespread and destructive diseases of peas, particularly in North America, New Zealand, Europe, and Japan. Despite good cultural practices, the disease remains very destructive regionally and no effective fungicides to control this disease are available. No commercial varieties are available with high levels of resistance. However, germplasm with moderate to high levels of resistance have been reported by the USDA within introduced lines held in germplasm collection centers (Kraft and Pleger, 2001). Resistance is quantitatively inherited, and QTL markers have been developed to assist selection for improved resistance.

Fusarium root rot can be a serious root disease, mainly in North America and Europe, and is potentially very destructive in both irrigated and dryland farming systems. Currently no commercial cultivars are resistant to Fusarium root rot. Germplasm with partial resistance are being identified, and breeding effort is leading to the development varieties that are more tolerant (Kraft and Pleger, 2001).

Fusarium wilt has been reported in all countries where peas are grown and can be particularly severe where short rotations are practiced. Six races have been described for which genotype resistance is available in commercial varieties. The only economic way to control wilt is to plant resistant varieties. Regionally, breeders have primarily focused on incorporating required race resistance genes; however, varieties are also being

developed with more horizontal resistance. Pythium rot or damping off diseases tend to occur in regions where soil moisture is high and soil temperature is between 18 and 24°C. Genotypes that produce anthocyanin pigmented seed coats are generally more resistant.

3.4.5.1.2 Foliar Fungal Diseases

Ascochyta blight in field pea is a complex of three diseases: *Ascochyta pisi*, which causes leaf and pod spot; *Mycosphaerella pinodes*, the perfect stage of *A. pinodes*, which causes blight; and *Phoma medicaginis* var. *pinodella*, which causes root rot. These diseases are widespread wherever peas are grown and are particularly a problem where peas are grown over mild and wet growing conditions, such as Australia. There are many reports of resistance within wild types. However, the genetics of resistance to these diseases are complex and poorly understood. This is mainly a result of the difficulty in distinguishing resistant and susceptible reactions. Studies suggest that resistance to *M. pinodes* and *A. pisi* is a result of hypersensitive reactions (Clulow et al., 1991). Several escape mechanisms have been identified, which have practical benefits to variety development in some regions, such as phenology (e.g., plants become increasingly susceptible as they mature) and multibranching (e.g., plants compensate for primary branch damage). Specific genotypes can also yield well under high disease pressure, and these differences in tolerance can be easily selected for and have the most practical value to breeders where the disease is severe (Armstrong et al., unpublished results).

Powdery mildew occurs in all regions of the world. The disease can cause severe damage if it occurs early in the season, prior to flowering. It is most prevalent in subtropical regions and in temperate areas where vegetative growth is extended by late sowing or later than average rainfall. Resistance to powdery mildew is inherited mostly as a recessive gene *(er1)*, which has provided breeders with complete and durable resistance (Tiwari, 1997).

Downy mildew occurs in periods of cool moist weather and is widespread. Regionally, symptoms can vary from local to systemic (i.e., whole plant). Three genetic mechanisms for resistance are recognized. While sources of resistance have been identified, differences in pathogen virulence also exist. Field and glasshouse assessment provide breeders with an indication of the level of partial resistance rather than full resistance, as most varieties are considered to be susceptible under high disease pressure. Wrinkle seeded vining peas appear to be more susceptible under high disease pressure in Europe than dry peas (Kraft and Pleger, 2001).

Other more regionally important foliar fungal diseases includes Sclerotinia white mold, Botrytis grey mold, Anthracnose, Septoria blotch, Pea rust, and Alternaria blight. Of these, practical partial resistance to pea rust has been identified and used in breeding in India. Variation in genotype resistance to Septoria blotch has also been reported in Australia, where it occurs sporadically (Leonforte et al., unpublished).

3.4.5.2 Viruses

Viral diseases are important in most areas of the world where peas are grown. Several resistance genes have been identified with practical benefit to breeders (Table 3.2). For pea enation mosaic,

Table 3.2 Major Viruses and Genetics of Resistance in Pea

Virus	Major Group	Resistance Genes
Pea enation mosaic	Enamovirus	En
Bean leaf roll	Luteovirus	Lr
Pea seedborne mosaic	Potyvirus	sbm
Bean yellow mosaic	Potyvirus	mo
Watermelon mosaic	Potyvirus	mo
Pea Streak	Carlavirus	Multigenic
Red clover vein mosaic	Carlavirus	Multigenic
Alfalfa mosaic	Alfalmovirus	Multigenic

Bean leaf roll, Pea seed borne, and Bean yellow mosaic viruses, resistance is an essential control strategy where these diseases are important.

3.4.5.3 Pests

Insects (e.g., wireworms, aphids, thrips, pea leaf weevil, pea weevil, loopers, cutworms) can cause severe damage to growing crop plants and grain. Genetic control mechanisms are currently not available for farming systems. The incorporation of pea weevil resistance from the related wild species *P. fulvum* into pea appears promising (Clement et al., 2002).

3.4.6 Improved Tolerance to Abiotic Constraints

3.4.6.1 Cold Tolerance

Autumn sown peas may be exposed to prolonged freezing temperatures and snow in some regions, whereas in regions with less severe winters, frosts are very damaging, especially at flowering. Air temperatures below −22°C were lethal, while fully hardened peas could withstand temperatures as low as −14°C (Eteve, 1985). Plant apices were more sensitive to frost after a period of relatively mild temperatures, with frost resistance enhanced by cold acclimatization. In flower buds, exclusion of water was associated with avoidance of freezing injury achieved by “preferential movement of water to the vegetative part of developmental nodes in the apex” under slow cooling of less than 2°C/hr. Leaf primordia help protect the apical meristem, and the “semi-leafless types are often more sensitive to freezing temperatures than conventional leaf types” (Eteve, 1985). Black hilum is closely linked with winter hardiness, along with the combination of high anthocyanin and purple flowers and prostrate growth habit (Eteve, 1985; Makarian et al., 1968).

Both controlled environments and multiple autumn field sowing to obtain coincidence of flowering with frost events have been used in screening for frost tolerance (Malhotra and Saxena, 1993). Autumn sown pea cultivars were reported as winter hardy (Belcher et al., 1999). Parents and progeny of both Melrose and Fenn varieties crossed with ID2 had low damage in laboratory screening at −6°C, with quantitative additive inheritance and correlation with field survival (Auld et al., 1983). In the same field trial, 14 *P. sativum* and 4 *P. sativum* subsp. *arvense* lines from a total of 370 screened had useful levels of winter hardiness. Balachkova et al. (1986) observed no damage to any *P. fulvum* accessions at −6°C, and though most *P. sativum* accessions were susceptible, a few conventional, plus a few short stemmed lines, were the most resistant.

Genetic variation for winter hardiness is available in both cultivated and wild pea, and this appears to be relevant to frost tolerance, unless preceded by mild or warm temperatures. Successful screening for these expressions has been demonstrated, and breeding of resistant varieties appears to be feasible.

3.4.6.2 *Soil Nutritional*

Both saline and sodic soils are common in West and Central Asia and in Australia (Brinkman, 1980). Saline soils are defined as having greater than 2 dS/m, while sodic soils have a high sodium absorption ratio and mainly have a pH above 9 (Bresler et al., 1982). Critical levels of salinity for growth effects on pea have been established, and screening procedures developed, to identify genetic tolerance (Saxena et al., 1993). Symbiotic nitrogen fixation is particularly sensitive to high salinity (Saxena et al., 1993), and screening for salinity may need to be done with and without a supply of mineral nitrogen. Genetic variation in salinity response has been documented in cultivars and lines of *P. sativum*, *P. elatius*, and *P. fulvum* (Poljakoff-Mayber, 1981; Cerda et al., 1982).

One mechanism of tolerance may be associated with antioxidant defenses (Hernandez et al., 2000), but exclusion of uptake of ions of both sodium and chlorine, and regulation of salt accumu-lation by cellular organization, are also cited as mechanisms (Saxena et al., 1993). Field environments are generally too variable for screening purposes, and controlled nutrient supply is recommended.

Soils with toxic levels of boron occur in southern Australia, and cultural techniques for screening tolerance have been developed for peas (Bagheri et al., 1992). The most tolerant cultivars were the ones most widely grown, including the traditional Dundale. These cultivars had less reduction in dry weight and lower concentration of boron as test levels of soil boron were increased. The inheritance of boron tolerance was attributed to two major loci with incomplete dominance, which interacted in an additive manner (Bagheri et al., 1996). In comparison with boron-tolerant varieties from other regions, RAPD analyses showed divergence of these with new Australian varieties, and a backcrossing program was suggested to transfer boron tolerance to a locally adapted genetic background. These studies have resulted in development of boron tolerance in pea as an objective in conventional pea breeding in southern Australia.

For disease, cold, and nutritional stresses, the wild relatives of *Pisum* may provide a rich source of genetic variation for improved stress resistances, particularly with

molecular biological techniques to develop markers and genetic maps, which enable targeted introgression of traits into cultivars (Muehlbauer, 1993).

3.5 NITROGEN FIXATION-GENETIC VARIATION IN HOST AND RHIZOBIA

3.5.1 Nitrogen Fixation and Pulse Production

Legume nitrogen (N_2) fixation plays a key role in the maintenance of world crop production. Legume N_2 fixation is a valuable process in world agriculture, contributing almost 20% to the nitrogen needs for world grain and oilseed production (Herridge and Rose, 2000). For all legumes, there is great potential to increase the percentage of legume N derived from N_2 fixation, as well to enhance the total N_2 fixed through improved management and genetic modification of the plant. The relationship between the legumes and their symbiotic N_2 fixing root nodule bacteria (the rhizobia) and how the symbiosis in the root nodules function to influence plant productivity and soil fertility are also considered.

The concept that legume N_2 fixation can be enhanced through selection and breeding has been recently reviewed (Herridge and Rose, 2000) with three general strategies proposed for increasing legume nitrogen fixation through breeding. Strategies of relevance to the expansion of field peas include maximizing legume biomass and seed yield according to the environment, agronomic management and the optimizing of legume nodulation, and enhancing the ability of the legume to nodulate and fix N_2 in the presence of soil nitrate.

Measurement of legume N_2 fixation in selection and breeding programs has included the following: N yield, N differences, ^{15}N, acetylene (C_2H_2) reduction, and xylem solute (ureide) methods (Unkovich and Pate, 2000). The proportion of legume N derived from the atmosphere by N_2 fixation (%Ndfa) for field pea using δ^{15} natural abundance methodology can vary between crops and soil environments. Field pea showed a lower proportional dependence on fixation and a lower mean net N benefit to following crops in southwest Australia when peas were grown in highly N deficient soils, compared with peas grown in eastern Australia (Unkovich et al., 1995). This lower net N benefit is likely due to the higher proportion of total N recovered in grain.

Estimates of N_2 fixation by individual crop species vary considerably between years and sites, often making it difficult to generalize about how much N a particular legume species can fix. Recent literature summarized by Unkovich and Pate (2000) showed the %Ndfa of field pea to vary between 5 and 95%, with the amount of N fixed in shoots to range from 4 to 244 kg N ha^{-1}. Under European conditions where world production is centered, 75% of total shoot crop N is supplied through N_2 fixation. Under Australian conditions where rainfall levels are lower, shoot N derived from N_2 fixation averages 68% (Unkovich and Pate, 2000). Total N_2 fixation of field pea can be underesti-mated by 40% if measurements are based on harvesting of leaves alone and omitting the below cutting height tissue (stolon and roots). The total amount of N_2 fixed by the whole plant (shoots plus roots) increased from 181 to 262 kg N ha^{-1}, much higher than the 121 to 175 kg N ha^{-1} obtained when only shoots were considered (McCallum et al., 2000).

In a symbiotic relationship, the plant is often regarded as the dominant partner in the symbiosis between the legume and the bacteria. No matter how effective, competitive, and persistent a strain of rhizobia might be, it cannot release its full capacity for N_2 fixation if limiting factors such as plant disease, nutrient deficiency, mineral toxicity (e.g., Al, Mn), salinity, unfavorable pH, weed competition, temperature extremes, insufficient or excessive soil moisture, inadequate photosynthesis, or the influence of grazing and management practices impose limitations on the vigor of the host legumes (Brockwell et al., 1995).

Soil temperature can affect legume nodulation and nitrogen fixation of cool season pulses. Legumes grown at low temperatures experience delays in the formation of nodules and the onset of nitrogen fixation (Peltzer et al., 2002). Even if background rhizobial populations are present, this delay in nodulation can impact on nitrogen fixation and ultimately affect growth and seed yield.

Legumes and rhizobia are both susceptible to soil pH outside their normal range. Acid soils provide an enormous challenge to rhizobial survival, the nodulation process, and establishment and functioning of the legume symbiosis (Dilworth et al., 2001). Poor persistence of *Rhizobium leguminosarum* bv *viciae* in acid soils is demonstrated by low nodulation score and poor plant growth (Slattery et al., 2003) and reflects the need for finding strains with more tolerance to acid conditions. On alkaline soils, background *Rhizobium leguminosarum* bv *viciae* populations persist well, with pulse production reliant on adequate soil moisture (Slattery et al., 2003). Reduced nodulation and nitrogen fixation can be evident in alkaline-sensitive legume species. Cool season pulses generally appear to be more sensitive to acidity than cereals (Siddique, 2000).

In legumes, mineral nutrients can limit nitrogen fixation through direct effects upon plant growth and the rhizobia, or through indirect effects upon the symbiosis (Slattery et al., 2001; O'Hara et al., 2003). The soil fertility impacts on N_2 fixation in Australian soils are primarily Al and Mn associated with soil acidity, P and Ca deficiencies on the less fertile soils, often those with low clay content and low cation exchange capacity. Boron toxicity is recognized as a widespread constraint to crop production, particularly in the low rainfall areas of southern Australia. Field pea appears to be relatively tolerant to B toxicity compared with lentil and chickpea (Siddique, 2000).

3.5.2 Rhizobial Selection and Development

Rhizobial inoculation is necessary with the expansion of pulses into a region with no previous pulse history, as few of the soils will contain sufficient background rhizobia. In hostile soil environments, the potential for N_2 fixation is limited, since rhizobia are often absent or, when present, ineffective. The current commercial strain in Australia for field pea is *Rhizobium leguminosarum* bv *viciae* strain SU303. This strain has been the inoculant strain since 1990, with a broad host range capable of infecting faba bean, lentil, and vetch plants (Unkovich et al., 1997). This bacterium may not be native to southern Australia, but it has shown to persist well in soils once introduced, although survival declines in soil where pH is less than 5 (Evans et al., 1993). Nodulation can continue throughout the life of the root system, with older nodules senescing as younger nodules form (Pate, 1977). N_2 fixation declines during later flowering, onset of high temperatures, and also during low water availability (Unkovich et al., 1997).

Certain characteristics are desirable for strains to be used as an inoculant. These include an ability to colonize the soil environment targeted for the host; competitiveness with the background populations; an ability to form effective symbiosis with the host legumes; a lack of deleterious effects on nontarget hosts; and genetic stability in culture, storage, and the soil (Brockwell et al., 1995; Howieson, 1999).

Considerable effort has been spent matching the root nodule bacteria to both the legumes and the intended soil environment (Howieson et al., 2000; Slattery et al., 2001). This step-by-step process illustrates a range of decisions required for consideration prior to initiation of a legume or rhizobial selection program. Improving the competitive ability of the inoculant strain improves successful colonization of plant roots, nodule formation, and then subsequently, N_2 fixation (Sessitsch et al., 2002).

3.6 MOLECULAR VARIATION

3.6.1 Molecular Variation within *Pisum sativum* ssp. *sativum*

Over the last decade, there has been an explosion in the use of molecular methods to assess intraspecific variation within *P. sativum*. The advantages over more conventional physiological or biochemical methods are that they are unaffected by the environment, independent of plant growth stage, and are able to be generated in large numbers that may cover a large area of the genome. This is particularly important for field pea since the crop is autogamous and very little phenotypic variation exists among closely related cultivars or accessions. Nevertheless, traditional methods used to determine genetic variation should not be discounted. For example, the electrophoretic pattern of dehydrins was assessed and two major mobility groups were identified among 12 *P. sativum* genotypes (Grosselindemann et al., 1998). Isozymes were subsequently employed to discriminate 45 commercial field pea cultivars, with a single isozyme profile able to distinguish 19 cultivars (Posvec and Griga, 2000).

However, with the advent of automation, the development, validation, application, and observation of variation with molecular methods is becoming highly reliable and relatively cost effective. The initial class of molecular tools used to assess variation within *P. sativum* ssp. *sativum* was Random Amplified Polymorphic DNA (RAPD) markers. RAPD markers are generated with the Polymerase Chain Reaction (PCR) using a single short (9 to 10 mer) oligonucleotide primer. Williams et al. (1990) demonstrated that RAPD markers are amplified from the entire genome, in both coding and noncoding regions and in single-copy and repeated sequences. The main advantages of this technique are that no prior knowledge of the genomic target sequence is required. Amplification product size differences (polymorphisms) are observed among individuals, usually on an agarose gel, and scored as present (1) or absent (0). A pair-wise binary matrix is then calculated among each pair of individuals using a genetic similarity coefficient (Li and Nei, 1979) to determine the variation that exists. Samec and Našinec (1995) were the first to show the power of molecular markers to discriminate field pea cultivars with very little phenotypic variation. For this, 59 polymorphic RAPD markers were observed and dissimilarity among economically important Czech *P. sativum* ssp. *sativum* cultivars ranged from 11 to 16%.

The RAPD technique is highly sensitive to reaction conditions and, hence, has a reputation of suffering from a lack of reproducibility among laboratories. However, this may be overcome by performing control reactions with standard genomic DNA and by using computer-automated PCR profile readers, to omit reliance on faint or irreproducible markers. Indeed, the RAPD technique is still being employed to determine variation and genetic relationships among field pea cultivar collections around the world. Recently, the pair-wise variation was revealed among 21 German cultivars with 175 polymorphic RAPD markers and ranged from 6 to 20% (Simioniuc et al., 2002). This study also assessed variation using Amplified Fragment Length Polymorphism (AFLP) markers, another marker class generated by the PCR reaction following a restriction digest and adapter primer ligation. The variation detected among the same 21 cultivars with 462 AFLP markers ranged from 6 to 15% (Simioniuc et al., 2002). A larger number of polymorphic markers were detected among the field pea cultivars using RAPD analysis (58%) than were detected using AFLP analysis (49%). In another study RAPD markers were able to discriminate all of the 10 pea genotypes, whereas the AFLP markers were not able to discriminate between two near-isogenic lines (Lu et al., 1996).

Another source of molecular variation that may be assessed among individuals was detected within the abundant and often degenerate DNA repeat sequences of the field pea genome (Turner and Ellis, 1997). Repetitive sequence was proposed to evolve at a higher rate due to “slippage” in the pre-transcription binding event and therefore, provides a good target to discriminate closely related individuals. Furthermore, due to long and sequence-specific primers, STMS markers are highly robust, transferable (Pandian et al., 2000), and allele specific. Burstin et al. (2001) retrieved 171 short sequence repeats (SSR) of *P. sativum* from the published Genbank/EMBL databases. Flanking primers were designed to 43 of the SSR sequences and used in PCR reactions to amplify sequence tagged microsatellite site (STMS) markers to determine variation among 12 field pea genotypes. The STMS markers revealed three groups among the genotypes: those collected from Afghanistan, the spring-type peas, and the fodder-type peas. Due to the long breeding history of the crop, the pedigree of field pea cultivars is often unknown. However, Burstin et al. (2001) demonstrated the use of STMS markers to elucidate potential parental lineages. Recently, an abundance of field pea locus-specific microsatellite primers were generated through the construction of a genomic library enriched for SSR motifs (Agrogene® consortium, France). Several of these primers were employed to study the variation among commonly grown Australian cultivars (Ford et al., 2002). A maximum variation of 22% was observed among the 15 pea genotypes assessed, and a preliminary DNA fingerprint database was developed using markers amplified from four of the STMS primer pairs. However, three groups of accessions could not be discriminated due to the amplification of identical allele sizes, demonstrating a potential genetic bottleneck in a field pea breeding program.

Molecular variation was studied within the field pea cultivar core collection at the John Innes Centre using sequence specific amplified polymorphisms (SSAP) in the Ty1-*copia* retrotransposable element (Ellis et al., 1998). The SSAP markers were compared with AFLP markers on the same set of genotypes (Lu et al., 1996). AFLP markers revealed a 22% variation, whereas SSAP markers were far more informative, revealing as much as a 59% variation among the 15 cultivars. The informativeness of the Ty1-*copia*

retrotransposable sequence for diversity analysis was reportedly due to its structural conservation and high degree of insertion site polymorphism.

Most recently, molecular technology has migrated into a new frontier where researchers and breeders alike are able to assess molecular variation through observing differences in the expression of functionally important sequences.

3.6.2 Molecular Variation among *Pisum* Subspecies and Species

Knowledge on inter-subspecific and interspecific genetic variation and relatedness in *Pisum* is sought for taxonomical classification and to determine genotypes for use in wide hybridization. Samec and Našinec (1995) used RAPD markers to separate *P. sativum* ssp. *sativum* and *P. sativum* ssp. *arvense* accessions. The maximum amount of inter-subspecific pair-wise dissimilarity detected was 17%. This was only marginally more than that detected within the *P. sativum* ssp. *sativum* group (16%) and indicated the close genetic relatedness among the two subspecies. In the subsequent study, cultivars of *P. sativum* ssp. *sativum* were compared against more cultivars of ssp. *arvense,* lines of ssp. *elatius,* and lines of ssp. *humile* (Samec and Našinec, 1996). Total variation among genotypes ranged from 3 to 51%, and the clustering in the dendrogram demonstrated that ssp. *arvense* was the closest to ssp. *pisum* with an inter-subspecific variation ranging from 12 to 31%. The ssp. *elatius* and ssp. *humile* lines were clearly separated from the cultivars with more inter-subspecies variation detected among them (51% maximum). The power of RAPD markers to assess variation, discriminate among taxa and determine taxonomy of the genus *Pisum* was further demonstrated by Hoey et al. (1996). Using 38 RAPD markers, the subspecies *elatius* was shown to be more closely related to the *P. sativum* cultivars (ssp. *sativum)* than the subspecies *humile,* which was also placed within the monophyletic clade. This indicated that accessions of previously separated northern (standard *sativum* karyotype) and southern (same chromosomal translocation as ssp. *elatius)* ssp. *humile,* ssp. *elatius,* and ssp. *sativum* belonged to the same species, *P. sativum*. Whereas, accessions of *P. fulvum* were placed outside the *P. sativum* clade and clustered as a separate species. These genetic relationships were further supported with the addition of data from 16 morphological and allozyme markers (Hoey et al., 1996).

More recently, Ellis et al. (1998) used polymorphic SSAP markers amplified from a Ty1-*copia* class retrotransposable element to conclude that *Pisum* comprises three main groups: *P. fulvum, P. abysinicum,* and all other *Pisum* ssp. Pearce et al. (2000) subsequently examined polymorphisms found within four Ty1-*copia* class retrotransposable elements to predict the taxonomical relationships within *Pisum*. Based on 446 sequence differences, three main accession groupings were again identified; *P. sativum, P. abysinicum,* and *P. fulvum* together with *P. elatius*. The closer association found between *P. elatius* and *P. fulvum,* than between *P. elatius* and *P. sativum,* was in direct contrast to the findings of Hoey et al. (1996). However, Pearce et al. (2000) did note that as much variation was detected among *P. elatius* accessions as was detected between *P. elatius* and *P. fulvum*. Also, the *P. elatius* accession JI2201 clustered with *P. sativum,* indicating either misclassification or some inter-subspecific hybridity.

Sequence polymorphism within the internal transcribed spacer (ITS) regions, found between the 18S, 5.8S, and 26S nuclear ribosomal genes, has also recently been assessed to determine the genetic relationships within *Pisum*. Saar and Polans (2000) identified

only 18 polymorphic nucleotide bases (13 in ITS1, 5 in ITS2) upon which to determine taxonomic status, further demonstrating the close evolutionary relationships among *Pisum* taxa.

Table 3.3 Initial Explants and Modes of Regeneration via Somatic Embryogenesis in Pea

Initial Explant	Mode of Regeneration	Author and Year
Leaf	Callus—mediated SE* (not regenerated to plants)	Jacobsen and Kysely, 1984
Immature embryos or shoot apices	Callus—mediated SE	Kysely et al., 1987; Kysely and Jacobsen, 1990
Protoplasts of zygotic embryo axes	Callus—mediated SE	Lehminger-Mertens and Jacobsen, 1989
Immature embryos	Direct SE	Tétu et al., 1990
Immature cotyledon	Callus—mediated SE	Özcan et al., 1993; Nadolska-Orczyk et al. 1994
Shoot apices	Direct SE	Loiseau et al., 1995; Griga, 1998

*SE—Somatic embryogenesis

3.7 TISSUE CULTURE AND GENETIC TRANSFORMATION

3.7.1 Somatic Embryogenesis

In vitro culture techniques such as transformation rely on the capacity of plant tissue to regenerate into whole plants, usually via somatic embryogenesis. Somatic embryogenesis is the initiation and development of embryos from cells that are not products of gametic fusion. Like zygotic embryos, somatic embryos have a shoot and root pole, enabling germination into complete plants (Karp, 1995). Somatic embryogenesis is particularly useful for the rapid production of large numbers of plants and is also widely used for the regeneration of transformed tissue. In the absence of a system of nucleo-cytoplasmic male sterility, somatic embryogenesis can also be utilized for the production of artificial seeds to enable the exploitation of hybrid vigor (Bencheikh and Gallais, 1996b).

Regeneration via somatic embryogenesis has been achieved from a number of different pea explants (Table 3.3). Shoot apices and immature zygotic embryos are now routinely used as initial explants (Kysely and Jacobsen, 1990; Loiseau et al., 1995). Somatic embryos can arise in two ways, either directly from the meristematic plant tissue, or indirectly from callus. The direct pathway is preferred for the development of clonal material, whereas the callus pathway is useful for the generation of large embryo numbers and for the induction of genetic variation in clonal regenerates.

As in many other species, success in generation of pea somatic embryos is highly dependent on the donor plant genotype. It is generally accepted that the competence for somatic embryogenesis is genetically controlled and mediated by the ontogenetic state of

the initial explant (Griga, 1998). Pea genotypes Cl-830 (Loiseau et al., 1995; Bencheikh and Gallais, 1996a) and HM-6 (Stejskal and Griga, 1992; Griga, 1998) were highly responsive to induction of somatic embryogenesis. Significant quantitative variation has been observed in inheritance of the ability to form somatic embryos, predominantly due to additive gene effects. Analyses of progeny from crosses between responsive and unresponsive genotypes of pea indicate that a few major genes control response to somatic embryogenesis (Bencheikh and Gallais, 1996b).

3.7.2 Somaclonal Variation

Somaclonal variation is the term coined by Larkin and Scowcroft (1981) to describe mutations that arise in leaf, stem, root, or other somatic tissues during plant cell and tissue culture. The exact mechanism of somaclonal variation is not fully understood, but the genetic background of the explant, the explant source, the medium composition, and the age of the culture can affect the frequency of variation. Somaclonal variation is probably caused by changes in the karyotype of the plant, affecting changes in number and structure of the chromosomes as well as chromosome rearrangements. Somatic gene arrangements, somatic crossing over, sister chromated exchange, deoxyribonucleic acid (DNA) amplification and deletion, transposable elements, DNA methylation, and epigenetic changes have also been observed (Brar and Jain, 1998).

Somaclonal variation can be exploited for improving genetic variability, especially in highly adapted genotypes lacking in a few key characteristics. These genotypes are induced to form callus or cell suspension cultures for several cycles, then regenerated in large numbers, generally via somatic embryogenesis. The resulting plants and their progenies are screened for desirable traits. *In vitro* selection can be used to determine agronomically desirable somaclones, particularly where cellular and whole plant responses are correlated (Sorboleva, 2001). *In vitro* selection for disease and herbicide resistance is also possible. For example, the addition of culture filtrate of the pathogen *Ascochyta pinodes (Mycosphaerella pinodes)* to culture media for the *in vitro* development of pea immature embryos enabled selection of tolerant pea germplasm (Kosturkova et al., 2001). Selected stable variants can be multiplied to develop new breeding lines.

Somaclonal variation in pea generated either directly via organogenesis, the process of *de novo* organ formation, or indirectly via callus-mediated somatic embryogenesis has been observed by many authors (Stejskal and Griga, 1992; Cecchini et al., 1992; Griga et al., 1995; Bernardi et al., 1995,1999; Ezhova et al., 1995; Sharma et al., 1996; Cavallini et al., 1996; Griga, 2000; Kosturkova et al., 2001; Weisner et al., 2001). Bernardi et al. (1995) demonstrated long-term callus culture of pea reduced the rDNA gene copy number, without changing the frequency of lectin and legumin genes. RFLP analysis indicated the occurrence of DNA rearrangements in rDNA genes and hypomethylation in the legumin gene. Weisner et al. (2001) reported qualitative alterations in cultured pea regenerates associated with sterility and lethality of primary regenerants. In addition, changes were observed in quantitative traits involving plant habit, yield parameters, and seed composition, with polymorphisms evident via random amplified polymorphic DNA (RAPD) analysis. Sharma et al. (1996) were able to select genotypes with a high degree of resistance to *A. pinodes* from the progeny of callus-derived somaclones.

The technique of somaclonal variation appears to be particularly important in enhancing variation in interspecific crosses, particularly where the parental genomes of the two species show little or no homeology. Under such situations, chromosome breakage and reunion could result in new combinations and in the transfer of alien chromosome segments into the cultivated species. The frequency of genetic exchange could be further enhanced by a tissue culture cycle of the hybrid material (Brar and Jain, 1998).

3.7.3 Transformation

Transformation, also termed genetic engineering, is the process of transferring isolated and cloned genes into the DNA, usually the chromosomal DNA, of another organism. Transformation of microorganisms was first achieved in 1973, but it was the early 1980s before successful plant transformation was reported, using the crown gall-inducing bacterium *Agrobacterium tumefaciens* to transfer genes for antibiotic resistance into tobacco. *Agrobacterium* has been called “nature’s own genetic engineer” because it uses a form of genetic engineering to introduce its own genes into plant cells. Plant genetic engineers use *Agrobacterium* strains disarmed of their plant gall-inducing ability as carriers or “vectors.” The modified vector is first transformed to carry the engineered gene constructs before being introduced into a host plant cell, where the new genes are integrated into the plant DNA. The *Agrobacterium* method has been widely adopted by researchers in many plant species as it is relatively simple and can be adapted to any laboratory with suitable tissue culture facilities (http://www.nuffieldbioethics.org/publications).

Transformation technology provides an opportunity to improve pea productivity via the introduction of traits from previously unavailable sources. Traits of interest are pest and disease resistance, improved protein quality, and herbicide tolerance (Nadolska-Orczyk and Orczyk, 2000). In addition to an efficient gene delivery system, successful transfer of foreign genes requires an effective selectable marker to indicate transformed cells, and the ability to regenerate fertile, transgenic plants via tissue culture. In pea, the β-glucuronidase (GUS) selectable marker has been widely used. This marker stains blue when exposed to 5-bromo-4-chloro-3-indolyl β-D-glucuronic acid (X-Gluc) solution, indicating areas where the gene has been incorporated. The regeneration of mature plants from a variety of explants, particularly via somatic embryogenesis, as discussed earlier, has enabled the use of various tissues as transformation target explants.

Early attempts at pea transformation resulted in a low level of plant regeneration and transgenic plants that were chimeric, sterile, or tetraploid (Nadolska-Orczyk and Orczyk, 2000). *Agrobacte*rium-mediated transformation was reported from stem explants (Lülsdorf et al., 1991), embryonic axis and epicotyl segments (Filippone and Lurquin, 1989; Puonti-Kaerlas et al., 1989), nodal explants (De Kathen and Jacobsen, 1990; Nauerby et al., 1991), and root explants and protoplasts (Schaerer and Pilet, 1991). No regeneration of mature pea plants was achieved from these reports.

Puonti-Kaerlas et al. (1990) were the first to achieve the production of mature, flowering, transgenic pea plants. They adopted regeneration from callus via organogenesis using a gene encoding hygromycin phosphotransferase as a selectable marker. Further refinement of the protocol by researchers at CSIRO, Australia (Schroeder

et al., 1993), and The John Innes Centre, U.K. (Davies et al., 1993) resulted in the much-improved production of phenotypically normal and fertile plants. Thanks to the work of these and subsequent researchers, *Agrobacterium-mediated* genetic transformation of pea has now become a relatively routine procedure in a number of laboratories worldwide. The most responsive target explants are the embryonic axis of immature seeds or the lateral cotyledonary meristems from germinating seed, and regeneration is generally via organogenesis.

Traits incorporated into pea via transformation include phosphinothricin resistance (Schroeder et al., 1993; Grant et al., 1999); kanamycin resistance (Davies et al., 1993; Grant et al., 1999); bruchid resistance via the αAl-1 gene (Chrispeels et al., 1998); resistance to pea enation mosaic virus (PEMV) via Plasmid pPCP4–5 (Chowrira et al., 1998); resistance to *Helicoverpa armigera* and *H. punctigera* via the protinase inhibitor cDNA (Na-PI) from tobacco (Charity et al., 1999); and resistance to pea weevil via α-amylase genes (Morton et al., 2000). As testimony to the routine nature of this procedure, the Legume Performance Group at the Plant Biotechnology Institute in Saskatoon, Saskatchewan, has inserted more than 40 gene constructs into pea using the *Agrobac*terium-mediated method (http://pbi-ibp.nrc-cnrc.gc.ca/en/research).

Other approaches to produce transgenic pea plants are: electroporation of protoplasts (Durieu et al., 2001), and particle bombardment of meristems. These techniques are not as widely used as *Agmbacterium-based* methods, due to their lower efficiency and the requirement for expensive and sophisticated equipment. Similar to other species, transformation efficiency depends on genotype, explant type, and other physical parameters.

In all currently used plant transformation methods, the transgene(s) cannot be directed to any particular point on the host chromosomes, resulting in more or less random incorporation into the host DNA. The location of the transgene in the host's DNA can determine its expression efficiency. Hence, it is often necessary to produce many individual transgenic plants to increase the probability of creating a genotype with all the desired characteristics. Another concern is the probability of transgenic traits outcrossing into conventional crops. Two years of confined transgenic trials by researchers at the Plant Biotechnology Institute, Saskatoon, Canada, have shown that transgenes could indeed migrate from transgenic peas into other pea varieties. However, the results also indicated that the transgenes did not travel far from their origin and that they were incorporated into other pea varieties with an overall low mean frequency of 0.065%. Hence, the Canadian researchers concluded outcrossing was unlikely to cause a threat to commercial pea production (http://pbi-ibp.nrc-cnrc.gc.ca/en/bulletin/2001issue3/page6.htm).

3.7.4 Interspecific and Intergeneric Hybridization

Interspecific hybridization is the crossing of two species from the same genus, and intergeneric hybridization is the crossing of two species from different genera. These techniques allow the exploitation of useful genes from wild, unimproved species for the benefit of the cultivated species. The *Pisum* species are diploid self-pollinators sharing a similar karyotype. All known wild accessions of *Pisum* are readily crossable to the cultigen (Muehlbauer et al., 1994). *Pisum fulvum* is the only separate wild species, and it

has been a useful source of disease and insect resistance. Fertile hybrids of *P. sativum*×*P. fulvum* have been produced via conventional unidirectional crossing with *P. fulvum* as the pollen donor, although a bridge cross with a wild *P. sativum* species is recommended to introduce *P. fulvum* genes into cultivated *P. sativum* (J.Wroth, personal communication, 2003).

3.7.4.1 Somatic Hybridization

The genetic variability of pea could also be increased by the introduction of genes from other genera via somatic hybridization, whereby genomes from different species and genera are combined without pollination through protoplast fusion. There are several reports of protoplast isolation from pea tissues (Lehminger-Mertens and Jacobsen, 1989, 1993; Puonti-Kaerlas et al., 1992; Ochatt et al., 2000). Most authors have reported callus regeneration, and some have obtained shoots (Puonti-Kaerlas and Eriksson, 1988; Lehminger-Mertens and Jacobsen, 1989; Böhmer et al., 1995)—and, more rarely, regeneration of whole plants via either embryogenesis or organogenesis (Lehminger-Mertens and Jacobsen, 1993; Ochatt et al., 2000).

The grass pea (*Lathyrus sativus* L.) possesses several interesting agronomic traits that might be useful for pea improvement, especially in respect to disease resistance (Campbell, 1997). Polyethylene glycol-mediated fusion of *Lathyrus* and *Pisum* has been reported, and heterokaryons have been produced (McCutchan et al., 1999; Durieu and Ochatt, 2000). Neither direct regeneration of whole plants nor indirect regeneration via callus has been reported from these heterokaryons. However, this technique holds promise as an alternative to transformation for the introduction of useful genes to pea from other genera.

3.7.5 Double Haploid Production

The development of homozygous, true-breeding individuals is one of the most time-consuming aspects of breeding self-fertilizing species and takes up to six generations in pea. This process can be accelerated via the development of doubled haploid breeding populations. In responsive species, haploid plants can be produced *in vitro* directly from the male or female gametes without fertilization. When the chromosome complement of these hemizygous haploid plants is artificially doubled, they become fertile doubled haploids, which are instantly and completely homozygous at each locus. A doubled haploid individual therefore has two identical homologs, and the amount of recombination information is equivalent to a backcross. The development of doubled haploid plants therefore achieves homozygosity in segregating populations in a single generation as opposed to five to six generations using a conventional breeding cycle. This enables selection of stable lines to start much earlier.

The ability to develop doubled haploid populations offers many opportunities for increasing genetic variation. Haploid production via isolated microspore culture, whereby immature pollen is cultured following isolation from the anther, is particularly useful for mutagenesis studies. The use of single microspore cells ensures genetic variation is expressed in the entire regenerated plant and, in some cases, allows for selection of mutants *in vitro* (Swanson and Erickson, 1989). The development of a haploid plant from

single cells also lends itself to the more efficient exploitation of genetic transformation techniques, such as microinjection, polyethylene glycol, *Agrobacterium* or particle bombardment. Doubled haploid-derived populations are also very useful for molecular mapping applications where homozygous individuals are extremely important. Due to the completely homozygous nature of doubled haploid material, it may be transferred among different laboratories and environments for assessing the effect of the environment on gene expression.

Pea, along with many of the large-seeded leguminous species, has traditionally been considered recalcitrant to *in vitro* haploid production. Recently, haploid embryos were produced from isolated microspores of pea, a first step in the development of doubled haploid technology (Lülsdorf et al., 2001). Canadian and Australian researchers continue to work on overcoming barriers to further embryo development and plant regeneration.

3.8 FUTURE DIRECTION

Molecular biology has added to the scope of plant breeding, principally in cereals, and with the full sequencing of the rice and arabidopsis genomes, the options for manipulation of plant expressions have dramatically increased. The most novel application is the capacity of interspecific genetic engineering to source either new gene expressions or modification of existing expressions from the whole array of life forms, for crop improvement. This process has barely begun, with asyet limited examples of transfer of pest and disease resistances to various crops and of vitamin A production in rice. There is great, but unrealized, opportunity to address all aspects of crop production, protection, utilization, and food and feed value.

Although most emphasis has been on the major cereal crops and certain dicotyledon species chosen for basic research, a transfer of such research by pea researchers can be expected in the future, albeit at a slower rate, reflecting current funding priorities. Importantly, the fundamentals for molecular breakthroughs in pea breeding are being developed. Successful tissue culture is a prerequisite for interspecific genetic engineering and is also a feature of double haploid acceleration of breeding in self-pollinated crops. Mapping with molecular markers and investigation of genome synteny across related plant families will allow both targeted genetic modification and increased efficiency in breeding for difficult-to-screen traits with marker-assisted selection.

For peas, the future directions may include excision of target genes from wild relatives into domestic peas with improved genetic backgrounds adapted to local conditions, sourcing of pest and disease resistance from distantly related or even unrelated plants and other organisms. Selection for improved nitrogen fixation may be feasible via specific compatibilities in both host and rhizobia genotypes and by marker-assisted selection for component processes in the interactive chain of genetic regulation of nitrogen fixation (Snoek et al., 2003). Some of these benefits may be long term, but they are worth flagging for future attention. Increasingly, research is targeted across species as in the CGIAR Challenge program for drought tolerance, which will include both cereals and legumes.

Commensurate attention to evaluation and exploration of the pea germplasm is also important. Before resorting to interspecific genetic engineering, the possibilities within

the pea germplasm need to be investigated. This requires more efficient gene search strategies such as the informed use of a core collection over the primary, secondary, and tertiary gene pools, which could extend to molecular candidate gene searches across diverse sources of intraspecific and interspecific DNA. Similarly, while conventional plant breeding will remain the basic approach, there will be many opportunities to slot in opportunistic molecular approaches, resulting in programs mixed for both molecular and traditional field screening of segregating progeny to realize new varieties.

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CHAPTER 4
Pigeonpea [*Cajanus cajan* (L.) Millsp.]

K.B. Saxena

Table 4.1 Regional Area, Production, Yield, and Compound Growth Rates of Pigeonpea

Region	1988–1990 Average			Compound Growth Rates (1970–1990)		
	Area (000 ha)	Production (000 t)	Yield (t ha^{-1})	Area (%)	Production (%)	Yield (%)
India	3555	2619	0.74	2.01	2.34	0.33
Other Asia	84	56	0.66	0.46	3.14	2.68
Africa	273	167	0.61	0.97	1.59	0.62
Latin America and Caribbean	62	46	0.74	1.58	−0.82	−2.40
World	3974	2888	0.73	1.88	2.25	0.37

Source: Adapted from Ryan (1996).

4.1 INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] occupies an important place among grain legumes due to its ability to grow under diverse cropping systems and environments and to recover from the losses caused by various biotic and abiotic stresses. The estimated global area of pigeonpea is more than 4 m ha, and the major pigeonpea growing countries are India, Myanmar, Nepal, Kenya, Malawi, Uganda, and Tanzania (Table 4.1). According to Ryan (1997), the global pigeonpea production trends largely reflect the situation in India, where area and production growth trends exceeded 2% a $year^{-1}$from 1970 to 1990.

In most breeding programs, besides increasing yield potential, the research has centered on understanding and alleviating important biotic and abiotic stresses. A significant breakthrough has been the shortening of maturity duration of the crop from the traditional 6 to 9 months to less than 3 months, which helps in the diversification of

cropping systems. Besides protein-rich food, this environment-friendly crop also provides much needed fodder and fuel wood in the dry areas. Its cultivation over a period helps in improving the soil fertility (Kumar et al., 1983) and its structure. Roots help in releasing soil-bound phosphorus (Ae et al., 1988) and make it available to the crop plants. Therefore, considering its ability to perform in diverse environments and systems and multiple-uses, pigeonpea appears to have a great potential in the tropics and semi-arid tropics of the world.

4.2 ORIGIN AND DISTRIBUTION

There are two opposing views on the center of origin of pigeonpea—one favors India, and another Africa. Considering the presence of a vast natural genetic variability in pigeonpea and presence of its wild relatives in the region, van Rheede (1686), Linnaeus (1737), and De (1974) supported an Indian origin. Recent excavation of pigeonpea seeds dated from the second century BC to the third century BC in India (Kajale, 1974) further strengthen this view. On the contrary, based on the exclusive distribution of *Atylosia kerstingii* Harnes, a wild relative of pigeonpea in Africa, and the reported discovery of a pigeonpea seed in Egyptian tombs of the XII Dynasty (2200 to 2400 BC) at Dra Abu Negga led Zeven and Zhukovsky (1975) and Brucher (1977) to support the African origin.

A wild relative of pigeonpea, *(C. cajanifolius),* which resembles pigeonpea in all morphological traits except the presence of a prominent strophiole, is considered to be the vital single-gene link between the cultivated and wild forms of pigeonpea (De, 1974). van der Maesen (1980) collected and identified *C. cajanifolius* in the forest of Central India. This important discovery has further strengthened the view that India is the native home of pigeonpea. Based on the genetic diversity within various species, he further concluded that (1) pigeonpea originated in India, (2) Africa is a secondary center of origin, and (3) Australia is an important center of diversity. The “Indian origin” theory has the most adherents and believes that the crop would have gone from India to Africa and Madagascar at least two millennia BC and to the new world along with the slave trade in postColumbian time (De, 1974). It is also postulated that pigeonpea moved from India via the Malay Archipelago to Indo-China and Australia.

4.3 TAXONOMY AND NOMENCLATURE

The first scientific name to pigeonpea, *Arbor trifolia indica,* was given by Bauhin and Cherla between 1650 and 1651 (van der Maesen, 1986), and the first binomial nomenclature to pigeonpea, *Cytisus cajan,* was given by Linnaeus (1753). *Cajanus indicus, C. flavus,* and *C. bicolor* are other binomials found in early literature. According to the International Rules of Botanical Nomenclature, the name finally adopted for pigeonpea is *Cajanus cajan* (L.) Millspaug. Pigeonpea has been classified in tribe Phaseoleae, subtribe Cajaninae, family Poaceae (Leguminosae), genus *Cajanus,* and species *cajan*. Within subtribe Cajaninae, there are 13 closely related genera, and among these, *Cajanus,* the only cultivated genera, was always considered to be genetically

related to one of the wild type *Atylosia*. The genus *Atylosia* W. & A. consists of 34 species, and they contribute to the secondary or tertiary gene pool. At International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), van der Maesen (1986) studied *Cajanus* and *Atylosia* genera and, based on various morphological, cytological, and chemo-taxonomical data, merged genus *Atylosia* into *Cajanus*. Consequently, the subtribe Cajaninae now contains 12 genera, and pigeonpea is the only cultivated food crop of this tribe.

According to De (1974), the popular Indian names of pigeonpea have been derived from the Sanskrit language, and *Adhuki or Adhuka* became *'Arhar'* while *Tuvarai* or *Tuvari* was later called *Tuar* or *Tur*. Some other names popular in different countries are 'Katjang' (Malaysia), 'Cguando' (Brazil), 'Gandolu' (Puerto Rico), 'Gungo' and 'Congo pea' (Jamaica), 'Quinchoncho' (Venezuela), 'Pois de Congo' (Africa), 'Kandulu' (southern India), Thora parippu' (Sri Lanka), and 'Mu dau' (China). It is called "red gram" or "pigeonpea" in English. The present-day internationally popular English name of this crop, pigeonpea, was coined by Plukenet (1692) in Barbados, where the crop was grown in barren lands primarily for feeding pigeons.

4.4 GENERAL BOTANY

Two prominent flowering habits, determinate and nondeterminate, are recognized in pigeonpea. In the determinate type, the apical bud of main shoot transforms into inflorescence, the flowering is more or less synchronous, and the flowers are borne in clusters at the top of a canopy. In the nondeterminate type, the apical bud is vegetative, and the floral clusters are borne in axillary racemes spread over considerable lengths of stem and branches. In the germplasm collection at ICRISAT, the number of days to maturity ranges from 85 to more than 280. Flowers of pigeonpea are predominantly yellow in color, with red streaks on the petals. Peduncles are 10 to 80 mm long. The pedicels are thin, 7 to 15 mm long, and covered with fine hairs. Bracts are 1 to 4 mm long, with a thick middle nerve and margins curved inward to form a boatlike structure. The calyx tube is companulate with glandular hairs and bulbous base, about 5 mm long, with 5 subequal triangular lobes. The corolla is highly zygomorphic. The petals are imbricate in the bud. The standard petal is either erect or spreading. Wing petals are obovate, 15 to 20 mm long, and about 6 to 7 mm wide. Keel petals are boat-shaped, glabrous, and split dorsally. Stamens are 10, diadelphous, 15 to 18 mm long, flattening toward the base and tapering toward the top. Anthers are ellipsoid, about 10 mm long, dorsified and yellow in color. The ovary is superior, 5 to 8 mm long, subsessile, densely pubescent, with 2 to 9 ovules. The stigma is capitate and glandular-papillate. The style is 10 to 12 mm long, filiform, and glabrous. The mature pollen grains are three-colporate with areolate orientation (Reddy, 1990). In nondeterminate pigeonpea, flowering extends for a few weeks, and thousands of flowers are produced, of which only about 10% set pods (Pathak, 1970). Fertilization occurs on the day of pollination and seeds mature about 40 to 50 days after pollination. In the first three weeks after fertilization, the pod wall grows more rapidly than the young seeds. The pod walls are known to contain various tannins. The ovules are arranged on a marginal placenta of the single carpell ovary. In each raceme, about 1 to 5 pods are formed. The pods of most pigeonpea varieties are

oblong, straight, or sickle-shaped; laterally compressed; green when young; strawcolored when ripe; and are often streaked to various degree with purple. Pod length varies from 20 to 80 mm, but rarely 130 mm (Reddy, 1990). The pods of most genotypes are nonshattering.

The most common seed shape is oval, but other types are also present in the germplasm. The common seed colors are brown and white, but other colors, such as purple, black, and various other combinations, are observed (Figure 4.1). The weight of 100 seeds ranges from 2.8 to 22.4 g. The average seed number per pod ranges from 2 to 9. However, the majority of the germplasm possess about 4 seeds pod^{-1}. The seeds are nonendospermic and contain two conspicuous cotyledons joined by gums. Strophiole is prominent on developing seeds, and it shrivels with maturity. Seeds do not have dormancy, and germination is hypogeal. The first trifoliate leaf emerges when the epicotyl is about 30 to 70 mm long. Secondary shoots, and sometimes multiple shoots, develop from the cotyledonary axils of the seeds in case the young plumules or axillary shoots are damaged (Reddy, 1990). In general, the large-seeded varieties produce bigger seedlings than those with small seeds, but such differences disappear as the plant attains growth (Narayanan et al., 1981). The first pair of leaves is simple, opposite, and caducous, but the subsequent leaves are compound, pinnately trifoliate, and arranged in a 2/5 type of spiral phyllotaxy. Leaflets are lanceolate or elliptic, with acute but sometimes obtuse apices (Reddy, 1990). Stems are ribbed, up to 150 mm in diameter, with prominent secondary growth. Most Asian lines have green stems, while African origin germplasm is characterized by purple color stems.

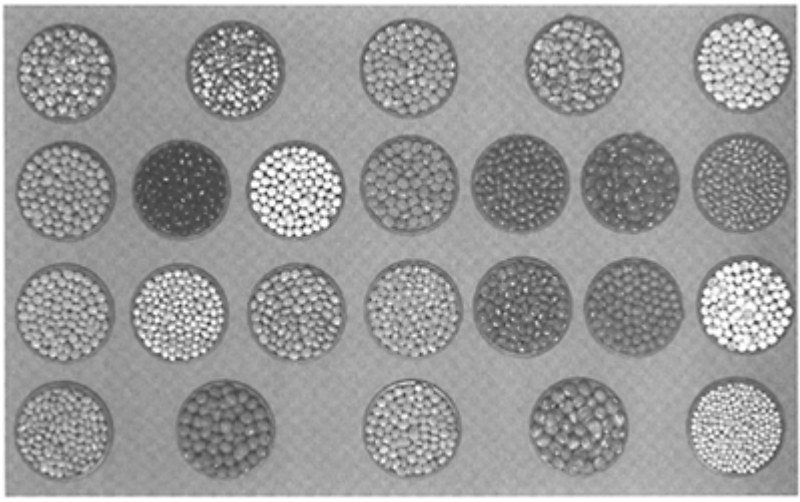

Figure 4.1 (See color insert following page 178) Seed color variation in pigeonpea.

Pigeonpea has a prominent tap root system with considerable lateral branching. The root growth begins shortly after sowing. The development of lateral roots occurs as soon

as the primary leaves begin to unfold. Due to the perennial nature of the plant, the roots continue to accumulate dry matter and produce laterals throughout the growth of plant until harvested or killed by diseases (Chauhan, 1993). The continuous production of new roots enables the plant to exploit the receding soil moisture in the post-rainy season. Under normal field conditions the roots may grow to a depth of 3 m. Tall, upright varieties produce longer and more deeply penetrating roots, whereas spreading types produce more spreading and dense root systems (Pathak, 1970). The roots of perennial pigeonpea grow up to 4 m in southern China (Saxena, 2002). Most roots feed within the first 600 mm soil profile. The primary structure of the root is usually tetrach and its secondary thickening takes place as a result of cambial activity (Bisen and Sheldrake, 1981). Pigeonpea is nodulated by Rhizobia of the cowpea group.

4.5 CYTOGENETICS

Roy (1933) while studying the female gametophytic tissues of pigeonpea reported the presence of 11 chromosomes. Krishnaswamy and Ayyangar (1935) studied the pollen mother cells and confirmed n=11, and they suggested that it was the basic chromosome number of the entire tribe. Naithani (1941) was the first to report 2n=22 somatic chromosome number. Akinola et al. (1972) examined 95 pigeonpea accessions of diverse origin and reported 2n=22 in the entire collection. van der Maesen (1986) observed that all of the 32 wild relatives carry the chromosome count similar to those of the cultivated types. However, in *C. kertsingii*, contrasting reports on chromosome numbers are found. Gill and Hussaini (1986) reported 2n=32, while Lackey (1980) recorded 2n =22 chromosomes; this large discrepancy needs to be verified.

Natural and induced tetraploids have been reported in pigeonpea. Pathak (1948), Pathak and Yadav (1951), and Saxena et al. (1982a) reported the existence of naturally occurring tetraploids (2n=44). Tetraploids characteristically have larger and thicker leaves and flowers with partial pollen sterility. Kumar et al. (1945) and Bhattacharjee (1956) reported colchicine-induced tetraploids. The partial male sterility and poor pod set observed in the tetraploid genotypes were attributed to varying degrees of multivalent formation and irregular chromosome disjunction during meiosis (Pathak, 1948; Bhattacharjee, 1956; Chopde et al., 1979). D'Cruz and Jadhav (1972) reported the first case of aneuploidy (2n=23). Anther-derived callus contained large variation in the chromosome number (2n=8 to 28) (Bajaj et al., 1980).

The somatic chromosomes are small, with the longest measuring 2.7 µm and the shortest 1.35 µm (Naithani, 1941). Deodikar and Thakar (1956) conducted the first detailed karyotype. A considerable genotypic variation was reported for total chromosome length (Sinha and Kumar, 1979) and satellited chromosomes (Sharma and Gupta, 1982; Pundir and Singh, 1986). One chromosome bivalent was attached with the nucleolus (Dundas et al., 1983; Reddy, 1981) while Kumar et al. (1987) reported two sites for nucleolar organization. The first detailed pachytene analysis of pigeonpea chromosomes was performed by Reddy (1981), and the chromosomes were characterized based on relative length, arm length, nucleolar association, and chromomere structure and distribution.

4.6 GERMPLASM RESOURCES

In 1973, ICRISAT was assigned the task of assembling pigeonpea germplasm on a worldwide basis to serve its own and other breeders across the globe. To assemble the germplasm, a number of field collection missions were undertaken in collaboration with various local institutions in 52 Asian, African, and Latin American countries. More than 13,548 pigeonpea accessions have been

Table 4.2 Phenotypic Variability for Different Traits Recorded in the Conserved Pigeonpea

Trait	Minimum	Maximum
Days to 50% flowering	45.0	237.0
Days to 75% maturity	85.0	299.0
Plant height (cm)	39.0	385.0
Primary branches (number)	2.0	66.0
Secondary branches (number)	0.3	146.0
Racemes (number)	6.0	915.0
Seed pod^{-1} (number)	1.6	9.0
100-seed mass (g)	2.8	25.8
Seed protein (%)	12.4	32.0

Note: Germplasm grown at ICRISAT, Patancheru.
Source: Compiled from various ICRISAT reports.

collected and characterized. Besides some cultivated and uncultivated landraces, these include wild relatives representing 47 species belonging to six genera. According to van der Maesen (1981), a reasonable coverage has been achieved from the Indian subcontinent—the primary center of diversity. He emphasized that the habitats of most wild relatives of pigeonpea are threatened due to increased pressure for expanding agricultural activities in new areas. Pigeonpea is of rather recent introduction in the Americas and the Pacific, and reasonably, representative collection is available. Van der Maesen further opined that in Africa and the Far East, pigeonpea has been grown for at least 4000 years. Significant genetic variability is expected from these regions, hence, more collection missions are needed.

The available germplasm collection at ICRISAT offers an extensive range of variability for most yield components and various morphological and quality traits (Table 4.2). In addition, pigeonpea contains a number of unique traits, such as genetic male sterility, cytoplasmic male sterility, modified floral morphology, dwarf, decumbent, single-culm, etc. A number of biotic and abiotic stresses play a major role in the adaptation and stability of cultivars. The germplasm collection contains 321 lines resistant to sterility mosaic, 29 to fusarium wilt, and 140 to P_2 race of phytophthora blight

disease. These valuable resources offer plenty of opportunities for developing varieties with single or multiple disease resistance.

In general, the pigeonpea landraces are heterogeneous for various traits primarily due to uncontrolled natural cross-pollination. This natural outbreeding produces a considerable genetic variability, and from such germplasm the breeders have identified some outstanding recombinants and a number of high-yielding popular varieties, such as C 11, BDN 1, Maruti, T 7, and UPAS 120. On the other hand, due to natural outcrossing, the maintenance and conservation of pigeonpea germplasm has become difficult and expensive. The botanists, however, try to conserve the available intra-accession variability by reconstituting the original population as close as possible. For this purpose, in each rejuvenation cycle one or two branches of individual plants of an accession are covered with muslin bags and 25 to 30 selfed seeds are bulked in equal number for storage and further use. The seeds can be safely stored under low temperature and low relative humidity. For long-term conservation at ICRISAT, the moisture content of seeds is brought down to about 5± 1% at 15% relative humidity, and the temperature is maintained at −20°C. The working germplasm, however, is conserved in a medium-term storage at 4°C and 20% relative humidity.

Secondary and tertiary gene pools are valuable resources for pigeonpea improvement. Many wild relatives such as *C. scarabaeoides, C. sericeus, C. lineatus, C. acutifolius, C. albicans, C. trinervius,* and *C. reticulatus* can be crossed (Table 4.3) with the cultivated pigeonpea and have been successfully used in the crop improvement programs. In comparison to the cultivated types, the wild species contain significantly higher amounts of seed protein. Accessions of *C. albicans, C. lineatus, C. sericeus,* and *C. crassus* have been found resistant to sterility mosaic disease. *Cajanus*

Table 4.3 Crossable Wild Relatives of Pigeonpea and Their Main Characteristics

Species	Habitat	Major Features	Reference
Cajanus	India, Sri Lanka,	Widely distributed in many countries; Antibiosis for pod borer; creeper climber; rectangular-rounded,	Roy and De, 1965
scarabaeoides	Australia, Africa	grey seeds with black mosaic	
C. sericeus	India	Erect shrub; 1 to 1.5 m tall, densely branched; grey or black rectangular-rounded seeds	Kumar et al., 1958
C. albicans	India, China	Perennial climber with woody base; rectangular, dark-colored seeds	Reddy et al., 1981
C. cajanifolius	India	Erect, open branches; 1 to 2 m tall; rectangular rounded seeds with black and grey color	Reddy et al., 1981

C. acutifolius	Australia	Erect or spreading shrub; 1 to 2 m tall, leaves covered with short silvery hairs; seeds dark and oblong	Dundas, 1984
C. lanceolatus	Australia	Erect, slender shrub; 1 to 3 m tall, seeds globose to compressed and dark mottled	Kumar, 1985
C. latisepalus	Australia	Erect shrub; 0.6 to 1.5 m tall, pubescent branches, seeds flattende-globose	Kumar, 1985
C. lineatus	India, Sri Lanka	Erect shrub; 0.5 to 2.5 m tall, open growth habit; seeds flattened-orbicular	Deodikar and Thakar, 1956
C. reticulates	Australia	—	Dundas, 1984
C. trinervius	India	Erect shrub; 0.5 to 2 m tall, stem and branches densely pubescent; seed dark colored and rectangular	Reddy et al., 1981

platycarpus is resistant to phyophthora blight, while *C. scarabaeoides* has shown a fairly high level of tolerance to Helicoverpa pod borer. Occasionally, some naturally outcrossed plants have been noticed in *C. scarabaeoides* and *C. sericeus*, but these wild species are generally maintained under natural conditions. A considerable genetic variation has been reported among the accessions of a wild species for various economic traits, such as disease resistance, seed protein, etc. (Saxena et al., 1990a). Therefore, to achieve rapid success in a breeding program involving exotic germplasm, a careful selection of a parent accession within a wild species is essential.

4.7 GERMPLASM ENHANCEMENT

In pigeonpea, where phenology is sensitive to environmental influences, a basic problem encountered in breeding for quantitative traits is the interpretation of results. Mating designs are influenced by physiological changes associated with phenological differences (Saxena et al., 1981). The estimates of important genetic parameters such as yield and its associated traits are also confounded with pleiotropic effects of genes influencing phenology. Saxena and Sharma (1990) reviewed the literature related to gene action and heritability of various economic traits in pigeonpea and reported low to high heritabilities and presence of both additive as well as nonadditive gene action for yield and major yield components such as pods plant^{-1}, seed size, seeds pod^{-1}, plant height, and days to flower and maturity. This suggests that for genetic improvement, besides pure line breeding, the population improvement and heterosis breeding programs can also be effective.

4.7.1 Agro-Ecological Adaptation

The adaptation of pigeonpea to different agro-ecological niches is governed by its duration, perennial nature, nondeterminateness, photothermal sensitivity of phenology, and susceptibility to biotic and abiotic stress factors. Photoperiod sensitivity has been reported to be associated with maturity (Wallis et al., 1981). The traditional medium- and long-duration cultivars are highly sensitive to photoperiod, and therefore, their adaptation is limited between 0 and 30°N or S latitudes. The

Figure 4.2 A short-duration variety of pigeonpea.

medium-duration cultivars are mainly grown between the Tropic of Cancer and the Tropic of Capricorn where winters are mild. In this range of latitudes, the long-duration cultivars generally suffer from terminal drought, except where rainfall is bimodal or temperatures are relatively mild due to high altitudes. The short-duration types are more or less insensitive to photoperiodic changes, and these can be grown successfully over a range (0 to 45°) of latitudes. This maturity group is receiving importance in breeding and agronomy research. A number of genotypes with wide adaptation have been bred (Saxena and Singh, 1996). Davis et al. (1995) reported that the short-duration pigeonpea cultivars bred at Patancheru (17°N) appear uniform under shorter day-lengths, but when tested under the long day-lengths of Minnesota (45°N), they were variable in flowering response. A further selection under long photoperiod environments resulted in the development of lines that were uniform for flowering in both environments. Such lines, when tested again in Patancheru, produced low biomass and yield and matured in only 85

to 90 days. Their biomass and yield, however, increased more rapidly than other cultivars under the artificially extended photoperiods as their maturity duration increased by 25 to 30 days (Chauhan, 2001). This suggests that the materials developed at higher latitudes can be grown successfully at lower latitudes, but the reverse may be difficult. Therefore, in order to develop cultivars adapted to particular latitudes, the day-length should be ideally longer than that prevails in the target location. The cultivars developed at higher latitudes under high temperature would flower too soon in climates with low temperatures and would not be able to accumulate sufficient biomass to produce high individual plant yield. This is indeed the case when Patancheru (17°N) bred material is taken to the highlands in Kenya. For achieving high yields in such environments, pigeonpea cultivars with long juvenile phase would be needed (Figure 4.2).

4.7.2 Natural Outcrossing

Howard et al. (1919) were the first to report 14% natural outcrossing in pigeonpea. Saxena et al. (1990b) reported a large variation (0 to 70%) in the extent of natural outcrossing in different genetic materials at diverse locations (Table 4.4). The large yellow- and red-colored flowers attract a variety of insects. These insects sit on the fully grown or open flowers and work on them. During

Table 4.4 Percent of Natural Outcrossing Recorded in Pigeonpea in Various Countries

Country/Place	Outcrossing (Range) (%)
India	
Pusa	01.6 to 12.0
Nagpur	03.0 to 48.0
Niphad	1 1.6 to 20.8
Ranchi	03.8 to 26.7
Varanasi	10.3 to 41.4
Badnapur	00.0 to 08.0
Coimbatore	1 0.0 to 70.0
Hyderabad	00.0 to 42.1
Kenya	1 2.6 to 45.9
Hawaii	05.9 to 30.0
Puerto Rico	05.5 to 06.3
Australia	02.0 to 40.0
Uganda	08.0 to 22.0

Source: Adapted from Saxena et al. (1997).

this tripping process, a load of pollen grains stick to the body of the insects, and when they visit other flowers and repeat tripping resulting in cross-pollination. Pathak (1970) reported *Apis mellifera* and *Apis dorsata* as the main pollinating agents. Williams (1977) reported a variety of insects visiting pigeonpea, but the *Megachile* spp. and *Apis mellifera* were found responsible for transferring pollen grains from one plant to another. She estimated there are between 5,500 and 107, 333 pollen grains on the body of each insect, and more than 90% of those pollen grains belonged to pigeonpea. In Kenya, Onim (1981) found 24 insect species visiting pigeonpea flowers, each visit lasted between 15 to 55 seconds, and that *Xylocopa* spp. (carpenter bee) and *Bombus* spp. (bumble bee) play an important role in cross-pollination. Brar et al. (1992) reported *Apis mellifera*, *A. dorsata*, *Xylocopa* spp., *Megachile lanata*, and *Ceratina binghami* visited pigeonpea flowers, but only *Megachile lanata* and *Apis dorsata* effected cross-pollination, while Verma and Sidhu (1995) reported high populations of *Megachile lanata* and *Xylocopa* spp. The large range in outcrossing reported in the literature might be due to a variety of combinations of various factors, including the number and type of pollinating insects, wind velocity, habitat, and crop growth. The number and type of pollinating vectors, however, are the most important factors. In addition, genotypic variation in floral morphology, such as tightly wrapped petals (Byth et al., 1982) and cleistogamous flowers (Saxena et al., 1992a), record reduced outcrossing.

4.7.3 Breeding Strategies

India accounts for more than 85% of global pigeonpea production, the national productivity ranges between 0.6 to 0.7 t ha^{-1}, and there has been no change in these statistics over the past few decades. This gives a dismal picture, and concerted efforts are needed to improve the yield levels.

Since pigeonpea is grown under a wide range of cropping systems and environments, and new systems have emerged, it is impossible to have similar breeding objectives, as such objectives may vary from one cropping system to the other, and approaches. The maximum gain in productivity will result from parallel advancement in both genetic and crop management. Since the phenology and estimates of genetic parameters are known to be highly sensitive to photothermal effects, and the existing production systems have evolved around them, breeding methods should address the core issues of the individual production and cropping systems, and therefore specific breeding programs need to developed (Byth et al., 1981). However, there are certain areas for which the breeding programs can be oriented across the environments and cropping systems. These include identification of the source of resistance for diseases and pests, transfer of a specific trait from the wild relatives, and various biotechnology related aspects. For a successful breeding program aimed at yield enhancement, a clear understanding of the physiological basis of yield and the genetics of important agronomic traits and their interrelationships under different ecosystems is essential. In comparison to cereals and other legumes, pigeonpea is relatively less researched and the available scientific knowledge is scanty in areas of physiology, breeding, genetics, and adaptation. Since additive as well as nonadditive genetic variances determine the expression of important agronomic traits, the breeding methodologies in pigeonpea should be geared to exploit them. The pigeonpea germplasm originating from Asia, Africa, and South America contain distinctly

prominent traits. For the improvement of yield and adaptation, hardly any attempt has been made to use this genetic wealth systematically, and most breeding programs suffer with this deficiency. Therefore, a targeted program for utilizing this genetic variation—and that from the crossable wild relatives of pigeonpea—will help in fulfilling the breeding objectives.

Three major maturity groups are recognized in pigeonpea. These are short (120 to 140 days), medium (180 to 200 days), and long (>250 days). Traditionally, the long-duration types are grown in northern India (27°N latitude) and Africa, while the medium-duration group is cultivated in Maharashtra, Andhra Pradesh, Karnataka, and Tamil Nadu in India and in Myanmar. The shortduration types generally have wide adaptability, and they can be grown in a range of environments. It has been observed that in several pigeonpea growing areas, the maturity of the cultivars does not match with the prevailing soil type, temperature, moisture availability, and cropping systems, which results in poor adaptation and yield. For example, in parts of southern India, long-duration types are traditionally grown, which suffer from high evapotranspiration and terminal drought at the crucial reproductive stage and yield ranges from 0.4 to 0.5 t ha^{-1}. Shifting to earlier maturing cultivars is likely to raise the productivity in these areas.

The subject of breeding varieties specifically suited for intercropping is always open for debate, largely due to a variety of crop combinations and their management practices. Green et al. (1981) summarized the results of a four-year trial and concluded that with 33% selection intensity only 55% of the highest yielding pigeonpea lines in intercrop with sorghum would have been selected from the pure crop. In all the pigeonpea breeding programs, selection and on-station evaluation of advance generation of materials is done as sole crop, while their final products are always grown under intercrop, as this may be the reason for low probability of success.

The natural outcrossing in pigeonpea is substantial, and it has significant influence on breeding efficiency. Since production of genetically pure seed in each generation of selection is practically impossible due to outcrossing, the gains from pedigree selection, especially for quantitative traits like yield, are limited. To overcome this limitation, Green et al. (1981) recommended the use of bulk hybrid advance, population breeding, and hybrid breeding. In the past, pedigree breeding has been successfully exploited for incorporating disease resistances, earliness, determinate growth habit, dwarfing, large seed size, white seed color, and long pod. On account of repeated natural outcrossing and vast segregation in each cycle of cultivation, invariably all the cultivars of pigeonpea have become highly heterogeneous for various qualitative and quantitative traits. This variability is considered a rich genetic resource pool from which useful variants can be selected. Shaw et al. (1933) were the first to recognize the value of this phenomenon and they identified 86 variants, of which some were highly resistant to fusarium wilt disease. A number of varieties have been developed from such inter- and intra-accession variability including popular cultivars like T 21, Prabhat, UPAS 120, C 11, BDN 1, HY 3C, HY 3A, T 7, LRG 30 in India, and ICEAP 00040 and ICEAP 00053, etc. in southern and eastern Africa.

Breeders are researching for any floral modification that would discourage natural outcrossing and produce seed with greater genetic purity. Two such variants have been identified. Abnormal free anthers and twisted petals characterize cleistogamous flower. Natural outcrossing ranges from 1 to 2% (Saxena et al., 1994). In other variants, where

the petals convolutely overlap, the natural outcrossing is also considerably reduced (Byth et al., 1982), but this trait is not dependable, as under certain environments a considerable degree of outcrossing takes place (Saxena et al., 1987b). The cleistogamous variant is easy to identify and simply inherited (Saxena et al., 1992a) and, therefore, recommended for developing new stable sources of resistance and pure line cultivars less threatened by genetic contamination under open pollination.

In conclusion, while developing a breeding program, consideration should be given to the prevailing cropping systems and agro-ecological parameters. Care should be taken to incorporate maximum genetic diversity in the selecting of parents for hybridization. Perhaps evaluation of potential parental lines for one or two seasons will be useful in achieving the optimum gains from a breeding program. The mating and selection methods should also consider the availability of human and financial resources.

4.7.3.1 Pure Line Breeding

4.7.3.1.1 Breeding for Seed Yield

Breeding for high grain yield and stability has long been the prime objective in most programs. Significant gain in yield has been achieved in pigeonpea by increasing area. However, the productivity of the crop has remained stagnant for decades. Considerable efforts have been made to improve the yield of traditional medium- and long-duration varieties through conventional breeding, but success has been elusive in most cases. Swaminathan (1973) attributed this failure to inefficient selection efficiency and various inherent physiological and management limitations of the crop. Chauhan et al. (1994) viewed it as the consequence of inherently poor partitioning of carbohydrates resulting in storage of high proportion of food reserves in nonreproductive parts and resulting in poor harvest index. The development of annual type may help in improving the harvest indices in pigeonpea (Saxena et al., 1992b).

Green et al. (1981) emphasized the influence of genotype-environment interactions on the manifestation of growth and yield in pigeonpea. They demonstrated that such interactions were particularly significant at macro and micro levels. They concluded that environmentally induced interplant variance is very high and, therefore, in early generation, selection for yield based on individual plants may not be effective. They recommended that the selection for yield must be based on progeny or family performance. To minimize the influence of different cropping systems and environments on selection, Byth et al. (1981) advocated that breeding activities should be targeted for each specific environment. Saxena and Sharma (1983) concluded that the low-yielding crosses can be safely rejected based on F_1 performance. High-yielding crosses in the F_1 should be tested in the F_2 as well for confirming the cross-performance and final selection, since the relationship between F_2 performance and that of later generations was more consistent.

In the short-duration group, which differs grossly in phenology and is cultivated as sole crop, a significant progress has been made in developing new high-yielding cultivars. The first highyielding short-duration variety, ICPL 87, was developed at ICRISAT from a single cross involving a long-duration large-seeded line and a short-duration small-seeded cultivar. The selections made in F_2 generation were handled

through the pedigree method. This variety produced a 10% higher yield than the control, with a 30% increase in seed size in the multilocation testing. This success encouraged breeders, and a number of varieties were released in this maturity group. They include ICPL 151, ICPL 2 (in India), ICPL 87091 (in Kenya, Malawi, and Uganda), Hunt, Quantam, Megha, and Quest (in Australia), and MN 1, MN 5, and MN 8 (in the U.S.). Since early maturity is associated with photoperiod insensitivity, these varieties showed relatively wider adaptation (Wallis et al., 1981). In the International Nurseries conducted by ICRISAT, such lines produced 2 to 3 t ha^{-1} yield (Table 4.5) at latitudes ranging from 0 to 42° N and S.

Saxena et al. (1986) demonstrated a quantum jump in the yield potential through heterosis breeding. The genetic male sterility-based hybrid ICPH 8 recorded 25 to 30% yield advantage in the farmers' fields (Saxena et al., 1992c). The development of more efficient cytoplasmic male

Table 4.5 Seed Yield (t ha-[1]) of Extra-Short-Duration Pigeonpea Lines at Different Latitudes 1988–89

	Latitude (°N)								
ICPL No.	**7**	**9**	**17**	**23**	**29**	**31**	**32**	**34**	**46**
83015	2.32	1.48	2.35	1.75	1.06	1.74	3.73	1.86	2.06
83019	2.21	1.39	1.46	1.43	1.00	1.36	3.58	1.67	1.76
84023	2.34	1.14	1.42	1.83	1.37	1.87	2.99	2.49	1.59
85010	2.79	1.55	1.59	1.88	1.17	1.25	3.16	2.33	2.15
85024	1.43	0.91	1.11	1.25	0.63	2.01	2.71	1.17	1.36
83006	3.09	1.51	2.22	1.49	1.28	2.73	3.38	2.00	1.43
85030	1.52	1.11	1.21	1.29	0.98	1.16	2.52	0.83	2.46
86010	1.67	1.08	1.86	1.78	1.80	1.20	3.47	1.23	1.33
Mean	2.17	1.27	1.65	1.59	1.16	1.67	3.19	1.70	1.77
SE	± 0.28	± 0.17	± 0.15	± 0.24	± 0.10	± 0.05	± 0.04	NA	NA
CV%	22.8	23.3	14.5	29.2	12.9	4.70	17.1	NA	NA

NA=Not available, single replication data.
Source: Saxena (2002).

sterility systems (Saxena and Kumar, 1999) has helped for greater gains in the productivity of this crop. This is discussed in greater detail in a later section.

4.7.3.1.2 Breeding for Disease Resistance

Breeding pigeonpea resistant to wilt, sterility mosaic, and phytophthora diseases has been attempted. Considerable success has been achieved in wilt and sterility mosaic resistance breeding, due to the development of an effective field screening technique. For developing a wilt-sick plot, sufficient inoculum is maintained uniformly in the field while for sterility mosaic disease, a simple spreader-row technique is used (Nene et al., 1981). Since both diseases are important from an adaptation point of view, a single nursery for both the diseases has been established at ICRISAT. The susceptible controls are grown at regular intervals to monitor the level of inoculums in the screening nursery in each season. The choice of breeding method for developing disease-resistant cultivars depends on the genetic nature of resistance sources. For wilt and sterility mosaic diseases, where good resistance sources are available, pedigree or mass selection has produced good results.

Pedigree selection within the landraces has been very effective in India and Africa for developing wilt resistant cultivars. In India, variety Maruti, a selection from ICP 8863 collected from Maharashtra State, is proving a boon to the farmers in the wilt-prone areas. In some districts, its adoption is as high as 60% (Bantilan and Joshi, 1996). A selection from a Tanzanian landrace (ICP 9145) has been adopted in 20% area of Malawi (Silim, 2001). Since wilt and sterility mosaic diseases may occur in the same region, ICRISAT emphasizes to breed varieties resistant to diseases together. Large F_2 populations involving single, double, or three-way crosses are grown in the multipledisease-sick nurseries. Individual plants resistant to wilt and sterility mosaic diseases are identified. To maintain their genetic purity, these plants are selfed with muslin bags. In subsequent generations, the evaluation for yield and other agronomic traits is done in disease-free fields, and the same material is sown in disease-sick fields to monitor the disease incidence.

In 1992, ICRISAT developed a widely adapted medium-maturing pigeonpea variety, ICPL 87119, through bulk-pedigree method. This variety demonstrated high level of resistance to wilt and sterility mosaic diseases and was also high yielding. In the All India Coordinated Trials, ICPL 87119 recorded 20% yield advantage over the control C 11. This variety is very highly popular in peninsular India.

For phytophthora blight, although considerable research has been done on standardization of glasshouse and field inoculation techniques (Kannaiyan et al., 1981), obtaining uniform disease reaction in field and glasshouse is still a problem (Reddy et al., 1990). The development of a number of pathogenic races has further complicated this work. From the world collection, only a few lines, such as KPBR 80 and ICP 9252, have been identified with field resistance to P2 and P3 isolates, and this resistance is expressed only in adult plants.

Alternaria leaf spot [*Alternaria tenuissima* (Kunzo ex. Pers.) Wiltshire] is a disease frequently observed in the late-sown crops and in certain agro-ecological areas such as eastern parts (Bihar, Orissa) of India. Two resistant lines, ICPL 366 and DA 2, have been bred. Onim and Rubaihayo (1976) selected UC 796/1, UC 2113/1, ICP 8869, and ICP 12792 lines resistant to Cercospora leaf spot in Uganda. Line ICP 9177, a collection from Kenya, has shown immune reaction to powdery mildew (Raju, 1988). Some of the powdery mildew-resistant accessions such as ICP 8862 and ICP 7035 also have resistance to wilt and sterility mosaic diseases. Such materials are good source for multiple disease resistance breeding.

Wild relatives of the cultivated species are known to sources of useful genes. At ICRISAT, successful search for disease resistance has been made in the wild relatives. These include C. *scarabaeoides* and *C. sericeus* for sterility mosaic, and *C. platycarpus* for phytophthora blight and cyst nematode. The transfer of resistance from the wild relatives needs more resources and should be restricted in cases where the resistance in the cultivated types is lacking. For example, the resistance for wilt and sterility mosaic diseases is available in the cultivated types, which should be preferred in breeding. On the contrary, some *C. platycarpus* accessions have shown high level of resistance to P_3 isolate of phytophthora blight (Saxena et al., 1996c), a trait not available in the primary gene pool. Mallikarjuna and Moss (1995) transferred the phytophthora resistance to cultivated type using the embryo-rescue technique.

Information on the genetic control of pigeonpea diseases is restricted only to major diseases. Pal (1934) was the first to report that pigeonpea wilt resistance was controlled by multiple factors. Shaw (1936) confirmed the presence of two complementary genes for resistance. Joshi (1957) and Pawar and Mayee (1986) concluded that a single dominant gene determined resistance to fusarium wilt. Resistance to sterility mosaic disease was reported to be controlled by four independent loci, consisting of two duplicate dominant genes and two duplicate recessive genes. The expression of resistance of at least one dominant allele at locus 1 or 2 homozygous recessive genes at locus 3 or 4 are essential (Singh et al., 1983). Sharma et al. (1984) reported that two genes govern the resistance to sterility mosaic with multiple alleles for resistance to sterility mosaic diseases. Srinivas et al. (1997) reported two nonallelic recessive genes for resistance to race-1. A single gene controlled the disease reaction with three alleles for the race-2. In case of phytophthora resistance, the information is available only for P_2 isolate, and a single dominant gene controls the resistance (Sharma et al., 1982). A single recessive gene controls Alternaria blight resistance (Sharma et al., 1987).

4.7.3.1.3 Breeding for Insect Resistance

In pigeonpea, the use of cultivars resistant to the major insects would be a significant step toward the successful management of this age-old problem. Sadly, the success has been limited, despite large resources invested in this area. Breeding for resistance has been attempted for *Helicoverpa armigera* and *Maruca vitrata* using open-field screening methodology at ICRISAT. These programs started with germplasm screening under nonsprayed conditions to identify sources of resistance. A brief account of the constraints and achievements associated with breeding for resistance to major insects is given below:

Helicoverpa resistance breeding

At ICRISAT, extensive research has been conducted in the last 25 years for identifying lines with high level of *Helicoverpa* resistance. No truly resistant genotype was identified from more than 10,000 accessions screened. However, a few accessions with relatively less pod damage, classified by some as “tolerant” and by others as “resistant” were identified. These results were not

Table 4.6 Pod Borer Damage and Seed Yield of Helicoverpa Tolerant Variety Abhaya and Control BDN 1

	Abhaya		BDN 1 (Control)	
Year/Trait	**Pod Damage (%)**	**Yield (t ha^{-1})**	**Pod Damage (%)**	**Yield (t ha^{-1})**
1984	49.0	2.27	76.0	1.83
1985	11.6	1.84	33.4	1.44
1986	22.5	1.05	71.4	0.58
1987	70.6	2.73	94.2	1.54
1988	19.0	1.48	48.1	0.89
Mean	34.54	1.87	64.62	1.25

Source: Compiled from various ICRISAT reports.

consistent in the expression of resistance level over seasons and locations. In addition, intra-accession variability for compensative reproductive regrowth following pest damage further adds to the low heritability of the pod borer resistance. The variable insect population pressure in different years has also been a complicating factor.

The field screening for *Helicoverpa* resistance has resulted in the identification of some genotypes with noticeable ovipositional nonpreference but a change in insect behavior under no choice conditions restricted its use in host-plant resistance breeding (Reed and Lateef, 1990). Antibiosis, another potential resistance mechanism against *Helicoverpa,* also could not be used effectively in breeding due to undesirable level of the chemicals. Since different plant types are equally susceptible to *Helicoverpa,* the "plant type" approach for reduced damage also did not work. The transfer of moderate level of resistance from germplasm to agronomically superior genotypes was successfully achieved through hybridization and selection (Table 4.6). The released pigeonpea variety 'Abhaya' in India, tolerant *Helicoverpa,* could not make any impact due to severe pod damage in the years of high insect pressure and its susceptibility to fusarium wilt disease.

Maruca vitrata resistance breeding

Maruca vitrata (Geyer) is a serious insect pest of tropical legumes. In Sri Lanka, yield losses due to *Maruca* damage in pigeonpea range up to 100%. Field screening of 271 short-duration accessions revealed a large variation for *Maruca* damage to flowers and pods. On average, the *Maruca* damage in determinate accessions was higher (66 to 75%) than that of nondeterminate (41 to 50%) accessions (Saxena et al., 1996b). To purify the genetic stocks, pedigree selection for resistance to *Maruca* damage was carried out for four generations of unsprayed field plots.

Some determinate and nondeterminate selections showed significant yield advantage over controls (Table 4.7). Under insecticide-free conditions in comparison to controls, the yield losses under nonsprayed conditions in the resistant selections were smaller (Saxena et al., 2002a). The resistance to *Maruca* was conditioned through yield compensation mechanisms. Poor larval growth and any other interference in the normal growth cycle of larvae feeding on the resistant line were possible reasons that may have contributed to resistance (Sharma et al., 1999). Saxena et al. (2002a) showed that by using the Maruca-resistant genotypes, it is possible to reduce the number of insecticide sprays for economic yields.

Breeding for Resistance to Other Insects

Although podfly *(M. obtusa)* causes considerable yield losses in long-duration pigeonpea, little efforts have been made to breed resistant genotypes. The resistant selections such as GP 3/3, SL 21/2, ICP 7151, and ICP 8102 were identified in germplasm screening, and they exhibit 5 to 10% damage under unsprayed field conditions, whereas in the susceptibles, up to 50% damage was recorded (Singh et al., 1990). Lal et al. (1986) suggested that by selection for shorter reproductive duration (less time from flowering to maturity), the podfly damage could be significantly reduced or escaped. So far, no breeding program has been undertaken to develop podflyresistant cultivars.

In Kenya, Omanga and Matata (1987) observed genotypic differences to pod-sucking bug (*Clavigralla* spp.) damage. The selections 423/85 and 423/20 identified from local landraces had some level of resistance to the pod-sucking bug. The research on this aspect was not pursued further, and this resistance needs to be stabilized and quantified before using in the breeding program.

Table 4.7 Performance of Pigeonpea Lines Selected for Resistance to the Legume Pod *Borer, Maruca vitrata,* Maha Illuppallama, Sri Lanka, 1996–1997 Rainy Season

Genotype	Days to Flower*	Days to Maturity*	Seed Yield (t ha^{-1})		Yield Loss (%)
			Sprayed	Unsprayed	
Determinate					
MPG 537-M1–2–1B	62	109	2.39	2.01	15.9
MPG 537-M1–2–5B	59	108	2.07	1.83	11.6
MPG 537-M1–2-M4	60	107	2.09	1.86	11.0
MPG 537-M1–2-M13	57	107	2.37	1.53	35.4
MPG 537-M1–2-M16	58	107	2.09	1.62	22.5
ICPL 87 (control)	63	119	2.36	0.60	74.6

Mean (n=15)	60	108	2.12		1.52	28.3
SE (var)	±1.4	±1.4		±0.23		
SE (spray)	—	—		±0.08		
SE (var↔spray)	—	—		±0.32		
Nondeterminate						
MPG 664-M1–2-M2	63	109	2.41		1.99	17.4
MPG 664-M1–2-M13	65	110	2.64		2.19	17.1
MPG 664-M1–2-M22	69	111	2.25		1.67	25.8
MPG 664-M1–2-M23	69	110	2.90		1.68	42.1
MPG 664-M1–2-M27	67	110	2.22		1.92	13.5
UPAS 120 (control)	66	115	2.32		0.670	68.9
Mean (n=15)	66	110	2.50		1.42	
SE (var)	±1.5	± 1.1		± 0.20		
SE (spray)	—	—		±0.08		
SE (var↔spray)	—	—		±0.29		

* Under unsprayed conditions.
Source: Saxena (1999).

4.7.3.1.4 Breeding for Dwarfness

Realization of yield potential of pigeonpea cultivars is often restricted due to the damage caused by a variety of insects. In the absence of insect-resistant varieties and effective integrated pest management schemes, it seems that any attempt to increase pigeonpea productivity is unlikely to make an impact without the use of chemicals. Most cultivars at reproductive stage achieve the height of 2 to 3 m, which does not permit effective chemical sprays for controlling the insects. The introduction of dwarfing genes in productive backgrounds appears to be a logical approach for effective insect management to maximize yields. Saxena and Sharma (1995) reported 12 sources of genetic dwarfs in pigeonpea. Of these, D_1 has been utilized to combine yield and dwarfness in short, medium, and long duration groups. The height in dwarf inbred lines ranged from 70 to 80 cm and produced acceptable yields. Further breeding and selection is required to develop stable and productive genetically dwarf cultivars.

4.7.3.1.5 Breeding for High Protein

Protein content of pigeonpea *dhal* (decorticated split peas) ranges from 20 to 22%. However, a number of wild relatives of pigeonpea have protein content up to 32%. Three to four genes control the genetic variation for protein content in pigeonpea (Dahiya et al., 1977). A strong maternal effect in determining the seed protein content was reported by Dahiya and Brar (1977). Durga (1989) reported that the protein content was under additive and complementary gene effects. A breeding program was launched to enhance the protein content by transferring high protein genes from the wild relatives, such as *C. scarabaeoides*, *C. sericeus*, and *C. albicans*. Since these species have a number of agronomically undesirable traits including hard small seeds, bushy plant type, and low yield, the breeding for combining protein with high yield and quality seed proved difficult and required large resources. In each segregating generation, large populations were grown and selections were made for these traits. Among the high protein agronomically superior lines, the seed size was correlated negatively (Saxena et al., 1987a) like in other food crops. From this gene pool a number of high yielding lines have been derived (Saxena et al., 2002b). It is estimated that by growing such lines in one hectare, about 350 to 450 kg crude protein could be harvested, with an advantage of 80 to 100 kg protein ha^{-1} over the standard control. Biological evaluation of these lines using rat-feeding trials showed that high protein genotypes were nutritionally superior to the control cultivar (Singh et al., 1990).

4.7.3.1.6 Breeding for Vegetable Types

Pigeonpea pods picked still green are an excellent vegetable. Green pod is normally picked when the seeds have reached physiological maturity—that is, when they are fully grown but just before they lose their green color. It is currently an important market commodity in the Caribbean, Africa, and a few areas of India. Vegetable pigeonpea can be an excellent substitute when green pea (*Pisum sativum* L.) is unavailable. Although vegetable pigeonpea is not normally as sweet as green pea, it is preferred by some consumers and is usually less expensive. The traditional vegetable type is late maturing, produces green pods for a limited period, and pods and seeds are characteristically large. ICRISAT has bred a number of short-duration vegetable type lines from which fresh green pods can be harvested for a larger period to increase farmers' profitability. One such variety, ICPL 87091, has become very popular in India, Africa, and China. The vegetable pigeonpea seed is more nutritious than the dry seed because it has more protein, sugar, and fat than the mature seed (Table 4.8). In addition, its protein is easily digestible. Green seed contains considerably lower quantities of the sugars that produce gas in the intestine (flatulence). However, there are fewer minerals in the green seed than in the mature seed (Faris et al., 1987).

Table 4.8 Comparison of Some Nutritional Constituents of the Edible Portion of Dry Pea and Vegetable Pigeonpea on a Fresh-Weight Basis

Constituent	Green Pea	Vegetable Pigeonpea
Chemical Composition (g 100 g^{-1})		
Edible portion (shelling %)	53.0	72.0
Moisture	72.1	65.1
Protein	7.2	9.8
Carbohydrates	15.9	16.9
Crude fiber	4.0	6.2
Fat	0.1	1.0
Mineral and Trace Elements (mg 100 g^{-1})		
Calcium	20.0	57.0
Magnesium	34.0	58.0
Copper	0.2	0.4
Iron	1.5	1.1
Vitamins (mg 100 g^{-1})		
Carotene (Vit. A 100 g^{-1})	83.0	469.0
Thiamin (Vit. B1)	0.1	0.3
Riboflavin (Vit. B2)	0.01	0.3
Niacin	0.8	3.0
Ascorbic acid (Vit. C)	9.0	25.0

Source: Faris et al. (1987).

Table 4.9 Pigeonpea Varieties Developed through Induced Mutations

Variety Name	Year of Release	Parent Line	Mutagenic Treatment	Trait Improved
Co 3	1977	Co 1	0.6% EMS	Yield
TT5	1984	T 21	Fast neutron	Yield
TT6	1985	T 21	Fast neutron	Seed size
TAT 10	1985	T 21	Fast neutron mutants	Maturity

			crossed	
Co 5	1985	Co 1	16 Kr-rays	Yield

In comparison with pea, vegetable pigeonpea takes longer to cook and is not as sweet—but is much more nutritious. On a fresh-weight basis, vegetable pigeonpea has a greater edible portion, more protein, carbohydrates, crude fibers, fat, minerals, and more of some vitamins than green pea. Particularly noteworthy is their very high level of vitamin A and C.

4.7.3.2 Mutation Breeding

The initial crop improvement activities using induced mutations in pigeonpea were targeted to study the effective doses of various mutagens and induced genetic variation for various morphological traits. So far, 73 pigeonpea varieties have been released for India. Of those, only five were released through mutagenesis (Table 4.9). For EMS treatment, (0.6%) was found effective and variety Co 3 was developed. Another variety Co 5 was developed using 16 Kr of gamma rays. Two varieties, TT 5 and TT 6, were developed using fast neutron treatment. Besides high yield, TT 6 has 25% larger seed than parental line T 21 (Pawar et al., 1991). A variety TAT 10 was developed by mating two mutant pure lines derived from fast neutron treatment. This variety is high yielding and matures about 30 days earlier than the control. Bhatia (2000) postulated that in the new millennium, the use of traditional mutagens in developing cultivars will be restricted and considering the potential of this approach, future mutation research needs to be directed toward improving more difficult characters such as root traits, nodulation, host-pathogen interactions, photo-insensitivity, apomixis, and release of gene silencing in transgenics.

4.7.3.3 Population Breeding

In the self-pollinated crops, conventional breeding imposes restriction on the chance of recombination rates, retains tight and undesirable linkages, and restricts the number of desirable alleles at various loci that can be accumulated in the selected line. Pigeonpea, a predominantly selfpollinated crop with varying degrees of outcrossing, has populations with homozygous balance. Khan (1973) advocated the formation of composites for maintaining genetic variability, recombination breeding, and the selection of single plants for conventional breeding. He also emphasized that after three to four generations of random mating, these composites can be improved by a suitable system to provide a heterogeneous population, which can be released to the farmers as an open-pollinated variety.

To increase recombination by intermating of genotypes, a population-breeding program based on the dual population system (Rachie and Gardner, 1975) was initiated at ICRISAT. In the dual population method, a parent with an easily observable recessive marker is used. The F_2 is grown in isolation and plants with the recessive marker are harvested. In F_3 plants with the dominant marker gene are harvested, and in the F_4 plants with the recessive marker are taken. This alteration ensures that only cross-pollinated

plants are advanced. This method did not produce good results. Onim (1981) tested two population improvement methods—stratified mass selection and mass selection with progeny testing in marginal rainfall areas. He reported 2 and 4% yield gain per cycle under stratified mass selection and mass selection with progeny testing, respectively. Singh et al. (1999) compared the relative efficiency of four population improvement schemes in increasing the variability for yield and its component traits to the base population. Mean and genetic variation increased in all the selection schemes. Among the different population improvement schemes used, genetic variability was greater in selfed progeny selection and lowest in half-sib progeny selection.

4.7.3.4 Somaclonal Variability

The first attempt to exploit somaclonal variations for the varietal improvement in pigeonpea was made by Chintapalli et al. (1997). They regenerated plants from cotyledon explants of a shortduration brown seeded variety, ICPL 87. The regenerated R_2 plants exhibited considerable variability for floral morphology, plant height, seed size, and seed color. Tissue culture produced both dominant and recessive mutants. The promising selections were grown in field for pedigree selection for various agronomic traits. Field evaluation of 15 selected somaclonal variants exhibited large variation for yield, seed size, and seed color. The best selection, ICPL 99073, had white attractive seeds, with 25% increase in seed size and 30% more yield (Saxena, 2002).

4.7.4 Hybrid Breeding—The New Promising Technology

The genetic improvement research on various aspects of pigeonpea began in 1914, and new varieties, production packages, and resistant sources were developed. These achievements helped in increasing area significantly, but during the last several decades yield has remained unchanged, around 0.6 to 0.71 ha^{-1}. This issue of yield plateau was considered a major challenge, and to achieve any breakthrough, plans were made to utilize genetically diverse germplasm including its wild relatives in hybridization programs in different mating and selection schemes. But the success in enhancing yield has been elusive. Unlike other legumes, pigeonpea flowers permit partial natural outcrossirtg, and this phenomenon was a bottleneck in maintaining purity of varieties. ICRISAT decided to make use of this limited outcrossing to exploit its hybrid vigor for developing highyielding commercial hybrids. The effectiveness of hybrid breeding technology in any crop primarily depends on (a) the availability of grower-friendly male sterility-based seed production technology and (b) the presence of economic level of hybrid vigor for seed yield. Therefore, our initial research aimed at generating the crucial information on these two aspects.

4.7.4.1 Genetic Male Sterility Systems

Reddy et al. (1977) examined 7214 pigeonpea accessions and identified a male sterile variant based on anther morphology and pollen production with translucent anthers. The tetrads during microsporogenesis did not separate and gradually disintegrated in this natural mutant. It was due to persistence of tapetum and the intercellular wall of the two

adjacent microspores (Reddy et al., 1978). Saxena et al. (1983) reported another source of genetic male sterility (GMS), characterized by brown and shriveled arrowhead-shaped anthers. Dundas et al. (1981) reported that male sterility was caused by breakdown of microsporogenesis at young tetrad stage, and it was accompanied by degeneration of the tapetum by vacuolation during the first division of meiosis. The anthers of GMS were completely devoid of pollen grains. However, male sterile plants set sufficient pods under open-pollination and thus established their potential in the hybrid breeding program. Each male-sterility source was found to be controlled by a single recessive gene.

The use of GMS poses practical limitations in the commercial seed production of hybrids, since it requires manual roguing of fertile plants from the rows of the female parent. This problem could be overcome to some extent if there were morphological seedling marker genes closely linked with the male sterile gene, as reported in lettuce (Lindquist, 1960) and watermelon (Watts, 1962). Singh et al. (1993) reported a possible linkage between translucent male sterile gene and temperature sensitivity. Under field conditions, they found that when the minimum temperature dropped below 10°C and the mean day temperature below 18°C, the male sterile plants shed all the floral buds,

Table 4.10 Plant and Seed Characteristics of Three CMS Lines Developed at ICRISAT Center, Patancheru

Trait	CMS 85010	CMS 88034	CMS 13092
Days to flower	64.0±0.32	77.9±0.61	135.5±0.38
Plant height (cm)	66.3±0.80	113.8±1.92	182.4±0.73
Primary branches	15.4±0.26	16.5±0.89	12.6±0.47
1 00-seed mass (g)	9.2±0.11	10.9±0.15	12.6±0.12
Seeds pod-1	3.2±0.03	3.1±0.06	4.9±0.03
Growth habit	Determinate	Indeterminate	Indeterminate
Plant spread	Semi-spreading	Semi-spreading	Compact
Flower color	Yellow with light red streaks	Yellow	Yellow with red streaks
Stem color	Green	Green	Purple
Pod color	Green with brown streaks	Green with brown streaks	Green with purple streaks
Seed color	Brown	Brown	White

Source: Adapted from Saxena (2001).

whereas in the fertile segregants, the pod setting was normal. Unfortunately, this important work was not continued.

4.7.4.2 Cytoplasmic Male Sterility Systems

The development of cytoplasmic male sterile (CMS) lines in pigeonpea would effectively overcome the seed production inefficiencies of GMS-based hybrids and their female parents. The first attempt to develop a CMS line in pigeonpea was made by Reddy and Faris (1981) by crossing a wild species *(C. scambaeoides)* as female parent with the fertile F_1 hybrid plants of *C. cajan* and *C. scarabaeoides*. The resulting BC_1F_1 was fertile, but in BC_1F_2, some male sterile plants were identified, but due to accompanying female sterility, this material was not pursued further. Ariyanayagam et al. (1995) crossed *C. sericeus* as a female parent with pigeonpea. The F_1 plant was partially male sterile and the subsequent backcross populations segregated for male sterility. The reversion of some plants from complete male sterility to full or partial fertility further complicated the selection and stabilization of this trait (Saxena and Kumar, 1999). Intensive selection and backcrossing for five generations, however, resulted in the identification of three stable CMS lines (Table 4.10).

Recently, CMS lines have also been developed from the materials derived from the population having cytoplasm of *C. scarabaeoides* (Saxena, 2002). This source has shown greater stability in the expression of male sterility over locations and seasons. The identification of some male sterile segregants in the populations of crosses involving *C. cajanifolius* has also been reported (Saxena, 2002).

Environmental Effects on CMS

Experiments conducted at ICRISAT have revealed that expression of male sterility in CMS lines derived from *C. sericeus* cytoplasm is influenced by environment. The factors responsible for this sex reversal are still unclear. In a recently conducted trial involving environment-sensitive CMS selections (Table 4.11), it was observed that the CMS lines expressed complete male sterility in the month of August, when sown in mid-June. However, in the month of September, when day length and mean temperature started declining, a proportion of the male sterile plants turned fertile and produced normal pods and seeds. It was also observed that the amount of pollen produced by these plants differed grossly from plant to plant. Further, toward mid-February, when day length and mean temperature increased, these “converted fertiles” reverted back to male sterility. Seeds produced from such plants give rise to male sterile plants without any abnormality. Similar environmental effects have also been recorded in some F_1 crosses made to study the fertility restoration.

Table 4.11 Phenotypic Expression of Male-Fertility Fertile and Sterile Plants in the Environment-Sensitive CMS Lines of Pigeonpea Evaluated at ICRISAT during Rainy Season 2000–2001

	Ratio of Fertile to Sterile Plants				
Line No.	**September 17**	**September 28**	**October 10**	**November 1 1**	**February 15**
103	0:14	3:11	9:5	9:5	0:14
111	0:17	6:11	6:11	6:11	0:17
114	0:12	5:7	5:7	5:7	0:12
115	0:14	8:6	8:6	8:6	0:14
120	0:16	8:8	8:8	8:8	0:16
121	0:34	27:7	27:7	27:7	0:34
122	0:26	9:17	9:17	9:17	0:26
131	0:27	19:8	19:8	19:8	0:27
132	0:26	20:6	20:6	20:6	0:26
133	0:21	13:8	13:8	13:8	0:21
134	0:26	15:11	15:11	15:11	0:26
135	0:26	15:11	15:11	15:11	0:26
137	0:27	10:17	10:17	10:17	0:27
140	0:21	11:10	11:10	11:10	0:21
141	0:17	6:11	6:11	6:11	0:17
142	0:24	12:12	12:12	12:12	0:24
143	0:15	4:11	7:8	7:8	0:15

Source: Saxena (2002).

The influence of environment on the expression of CMS and its fertility restoration is not uncommon in crop plants. Kaul (1988), while reviewing male sterility in crop plants, quoted a number of such examples from vegetable, cereal, and legume crops. Among the environmental factors, photoperiod and temperature have been reported to influence pollen sterility, microsporogenesis, tapetal development, and seed set. He concluded that the stability of expression is not only fertility restoration and male sterility gene specific, but it depends on the presence or absence of other genes.

4.7.4.3 Fertility Restoration of CMS Lines

Identification of genotypes, which, when crossed with CMS lines, produce fertile F_1 hybrids, is a vital component of a three-parent hybrid breeding technology. This activity was initiated soon after developing CMS lines, and of the 291 combinations tested, only 7 fully restored the male fertility. For identifying new fertility restorers, the germplasm resources available in ICRISAT's gene bank are being used. Since most germplasm accessions are heterogeneous due to partial outcrossing, the F_1 hybrid progenies segregated for fertility restoration and other agronomic traits. Pure lines with 100% fertility restoration have been developed through selection from such germplasm (Saxena, 2002).

4.7.4.4 Special Features of Hybrids

4.7.4.4.1 High Yield

Since in any pulse crop no commercial hybrid is available, the release of the world's first pigeonpea hybrid, ICPH 8, in 1991 is a significant milestone (Saxena et al., 1989, 1992c). This hybrid was developed by crossing a GMS line (MS Prabhat DT) and a fertile inbred line, ICPL 161. It has nondeterminate vigorous growth, high yield, and early maturity, and escapes drought and major diseases. Evaluation from 100 yield trials in different agro-ecological zones showed ICPH 8 to be superior to controls UPAS 120 and Manak by 30.5 and 34.2%, respectively (Table

Table 4.12 Zonal Weighted Mean Yields of the World's First Pigeonpea Hybrid ICPH 8 in Different Zones in India over the Years

Zone	No. of Years	No. of Trials	Yield (t ha^{-1}) ICPH 8	Yield (t ha^{-1}) UPAS 120	Yield (t ha^{-1}) Manak	% Increase Over UPAS 120	% Increase Over Manak
Northwest Plains	6	36	2.85	2.10	2.34	35.0	31.0
Central	4	30	1.56	1.16	0.93	32.9	52.5
Southern	4	30	1.42	1.22	1.26	23.6	27.3
Northwestern Hills	1	2	1.56	1.50	1.19	4.3	31.0
Northeastern Hills	1	1	1.68	1.15	—	45.6	—
Western	1	1	2.06	1.41	1.59	45.6	29.5
Overall Mean		100	1.99	1.53	1.35	30.5	34.2

Source: Saxena et al. (1996).

4.12). Punjab Agricultural University, Ludhiana, India developed pigeonpea hybrid PPH 4 in 1993 (Verma and Sidhu, 1995), which outyielded the control T 21 by a margin of 47.4% on the basis of eight multilocation trials conducted over two years. In the All India

Coordinated Trials, this hybrid exhibited 32% superiority over the best national check, UPAS 120. Another hybrid, CoH 1, was released in 1994 by Tamil Nadu Agricultural University, Coimbatore, India. It recorded 32% higher yield over control VBN-1 in 17 trials (Murugarajendran et al., 1995). Two more hybrids, AKPH 4104 and AKPH 2022, were released in Central India and they recorded 35 to 64% superiority over the controls.

4.7.4.4.2 Faster Growth Rate

Inherently, pigeonpea is a slow growing crop, particularly in the early stages of growth. This makes it a less efficient crop as far as competition with weeds is concerned. Hybrids produce seedlings with greater vigor. The differences in growth vigor begin to appear during the early seedling stage and become more pronounced with time. This attribute of hybrids makes them more suitable for sole cropping than varieties, as it enables them to establish quickly and utilize light and water resources more efficiently. It was observed that in comparison to the pure line cultivars, the 1-month-old seedlings of hybrid ICPH 8 produced 43.9% higher shoot and 42.8% higher root mass, and such differences were maintained subsequently. Increasing spacing from 60 cm×20 cm (83,000 plants ha^{-1}) to 75×20 cm (66,000 plants ha^{-1}) did not affect the yield (Saxena et al, 1996a).

In cereals and some legumes, a considerable proportion of the genetic variation in yield is accounted for differences in partitioning of photosynthates. By contrast, variation in pigeonpea yield is due to the differences in crop growth rates. Hybrids are higher yielding mainly due to their higher crop growth rates than the pure line varieties. The higher crop growth rates can be achieved by agronomic manipulations, such as increasing plant population and changing sowing time, but this does not necessarily result in increased yield because of its negative effect on partitioning. On the contrary, hybrids exhibit higher crop growth rates while maintaining their partitioning at least at the same level as that of pure line varieties (Chauhan et al., 1994) and, thus, produce high yield. Hybrids have also shown significant improvement in the density of pods and seeds per pod. Higher crop growth rates of hybrids eventually result in both higher biomass production and seed yield.

4.7.4.4.3 Greater Drought Tolerance

Traditionally, pigeonpea is grown under rainfed conditions and is subjected to both intermittent and terminal droughts. Short duration pigeonpea has lesser root mass than the traditional mediumand long-duration types and, thus, is more prone to this stress. Incorporation of tolerance to drought is an important requirement if short duration is to succeed under rainfed conditions. Screening for drought tolerance using a soil moisture gradient with line source sprinkler irrigation showed that hybrids performed well not only under optimum soil moisture conditions but also under drought stress, and that it might be related to the vigor of their root systems, which enables them to have a greater access to stored soil water (Saxena et al., 1996a). Lopez et al. (1996) attributed superiority of the hybrids to their increased ability to maintain relatively high water content than pure line varieties. It therefore appears reasonable to assume that in pigeonpea hybrids we may not only achieve higher yield potential but also a greater ability to adapt to drought, a trait that is otherwise difficult to improve through traditional breeding efforts.

4.7.4.4.4 Greater Adaptation

In most field crops, hybrids in general show wide adaptation and stability of yield across the environments. At most locations, the hybrid ICPH 8 outyielded the control, and it expressed greater stability in performance when compared across the environments.

Hybrids also promise to address disease control. A joint evaluation of wilt- and sterility-mosaicresistant, pure line cultivars and hybrids in disease-free and disease-sick fields indicated that in the disease-sick nursery, both the hybrids as well as inbred controls were comparable in disease reaction, with less than 1% wilt and sterility mosaic disease incidence in both groups. Large differences, however, were observed in the expression of hybrid vigor under disease-free and disease-sick conditions. Although a general yield reduction was observed in all the genotypes when evaluated under disease-sick field, the expression of heterosis for yield was three times greater than diseasefree conditions. Hybrid vigor conveys an extra degree of resilience that enables plants to produce under severe disease pressure when compared with nonhybrid cultivars. Lopez et al. (1996) also reported higher adaptability of hybrids in comparison to inbred cultivars. It is also postulated that, besides genetic factors, the ability of hybrids to produce greater root and shoot biomass throughout its growth cycle imparts greater ability not only to utilize greater amounts of available nutrients, but also to tolerate yield-limiting stresses such as drought, water logging, and disease resistance. The high level of recovery in yield from various stresses also contributes to increased adaptation of the hybrids.

4.7.4.5 Hybrid Seed Production Technology

4.7.4.5.1 Isolation Specifications

The technology of producing hybrid seed must be simple and user-friendly. The production package should contain appropriate agronomical operational, insect management recommendations and information on post-harvest handling of the seed. An efficient seed production system that could provide quality seeds at economically viable costs is the backbone of such technology. Since outcrossing in pigeonpea is affected by insects, a safe distance is recommended so that the pollinating insects, carrying pollen grains on their body, could not fly to and pollinate the flowers of other lines. This is essential to produce genetically pure seed. So far, no isolation distance study has been conducted using male sterile lines of pigeonpea, and it is assumed that the information generated on isolation specifications of pure line cultivars could be utilized safely in producing seeds of pigeonpea hybrids and their parents.

The recommended isolation specifications for pigeonpea differ considerably on degrees of natural cross-pollination. Ariyanayagam (1976), citing the Food and Agricultural Organization (FAO), recommended a minimum isolation distance of 180 m and a maximum of 360 m, while Agarwal (1980) recommended distances of 400 m and 200 m for the production of foundation and certified seeds, respectively. Faris (1985) suggested that, for quality varietal seed production, two varieties must be separated by at least 100 m, while a distance of 200 m between varieties is essential if the seed is to be used by breeders. In India, the seed certification standards fixed with regard to isolation distance for varieties are 200 m for breeder seed and 100 m for both foundation and

certified seed (Tunwar and Singh, 1988). The experience at ICRISAT suggests that the FAO specifications are suitable for both the production of hybrid seeds and for maintaining male sterile and pollinator lines.

4.7.4.5.2 Male Sterile Lines

It is essential that male sterile stocks be genetically pure for uniform expression of heterosis. For GMS systems, the lines must be maintained in the heterozygote form by harvesting seeds from male sterile *(ms ms)* plants pollinated by fertile heterozygotes *(Ms ms)*. To multiply male sterile lines, the seeds harvested from male sterile plants *(ms ms+Ms ms)* are grown in isolation. At flowering, at least one young bud from each plant is manually opened and its anthers checked for the presence or absence of pollen grains. The male sterile and fertile plants are tagged with different colored markers. At maturity, seeds are only harvested from the male sterile plants. Immature pods are removed from segregants plants, if necessary, to extend the period of pollen availability (Saxena et al., 1996a). For multiplying seed of CMS line, the female male sterile rows (A line) and their maintainer (B line) are grown in 6:1 ratio. Pod set on the "A" line is accomplished by insects, which help in mass pollen transfer from "B" line to "A" line.

4.7.4.5.3 Pollinators

The genetic purity of pollinators is also essential for uniform expression of hybrid vigor. To prevent genetic contamination, the pollinators must be grown in isolation and off-types, if any, should be rogued before flowering starts.

Generally, the full pod set is realized if one pollinator row is sown after every six male sterile rows. In the production of GMS-based hybrids, the first bud that appears on each plant in the female rows is examined, and the male sterile plants are tagged while the male fertile segregants are rogued before their flowers open. It is a time-bound operation, and if roguing is delayed, the quality of the hybrid seed is adversely affected. In the CMS-based hybrid program, the same (1 male:6 female) ratio is used, but in the female rows, identification and roguing operations are not carried out. This not only saves expensive labor but also helps in maintaining quality of the hybrid seed.

Since pigeonpea is a perennial plant, flowering on the male sterile plants continues until the potential number of pods are set on each plant. This could be due to lack of pollinating insects or nonsynchrony of flowering of the two parents. On the contrary, in the pollinator rows, the flowering terminates when the potential pod set is realized by selfing. To ensure adequate hybrid yields, flowering in the pollinator rows can be extended by periodically removing young, developing pods and frequent irrigation. In situations where the pollinating insects are inadequate or plant growth is variable, the recommended 1 male:6 female ratio may not be adequate for optimizing hybrid seed yield, and it may be modified to suit local conditions.

4.7.4.5.4 Economics of Seed Production

Seed cost plays an important role in the adoption of hybrids. In a detailed study conducted at Coimbatore using GMS system, 813 kg ha^{-1} of hybrid seed was obtained in

a single harvest, resulting in approximately 1:32 seed-to-seed ratio. The estimated cost of hybrid seed was Rs 21.87 (1 U.S.$=Rs 45) kg^{-1}. In this study, the roguing operation alone accounted for about 45% of the total production cost (Murugarajendran et al., 1990). In tropical environments with warm winters, pigeonpea produces several flushes of pods within a year, and the perennial nature of this crop can be exploited to produce quality hybrid seed at low cost by adopting multiple harvest systems (Saxena et al., 1992c). So far, no study has been conducted to determine the cost of hybrid seed production using the CMS system, but it is likely to be more economical than the GMS technology, because in the former the costly operation of roguing is eliminated.

4.8 TRADITIONAL AND NONTRADITIONAL USES OF PIGEONPEA

4.8.1 Food

Dhal (decorticate split peas) is the most acceptable form of pigeonpea consumption. It is made by dehulling and splitting the two cotyledons of dry seeds. In India, almost all pigeonpea produce (2.2 mt) is converted into *dhal* by more than 10,000 milling units scattered all over the country. White-colored, whole dry seeds of pigeonpea are used in various traditional foods and snacks in eastern Africa, western India, and Indonesia. In addition, a number of food items can be prepared from pigeonpea seed or flour. These include tempeh, fresh sprouts, ketchup, canned dry seeds, and various extruded food products, such as snacks, noodles, etc. Immature or green seeds of pigeonpea can be used as a vegetable. They can be grown in backyards—or commercially, in large fields. Green pigeonpea is an important vegetable in the Caribbean. In Puerto Rico and the Dominican Republic, green seeds are canned for local consumption and for export. Frozen and dehydrated green seeds are also consumed in some countries.

4.8.2 Animal Fodder and Feed

Pigeonpea plant and grains have been used as animal feed by Indian farmers for centuries. After harvesting pods, the plants are left in the field for browsing by domesticated animals. It produces about 20 to 25 t ha^{-1} of edible dry forage and provides fodder at a time of the year when there is a deficit of energy and protein for the animals (Whiteman and Norton, 1981). Leaves can provide a good substitute for alfalfa in animal feed formulations, particularly in areas that are not suitable for growing alfalfa (Embong and Ravoof, 1978). The recovery of *dhal* ranges from 70 to 80% during milling. The remaining portion, consisting of seeds, brokens, powder, and husks, produced as by-products, can also be used as animal feed. These are considered protein-rich concentrates for both ruminant and nonruminant animals.

4.8.3 Other Plant Products

Dried stems and branches provide excellent fuel for rural households in India. The heat value of pigeonpea wood is about half that of the same weight of coal (Panikar, 1950).

Their straight branches are used in villages for roofing, wattling on carts, baskets, and temporary fences. Pigeonpea can also be used to produce lac and silk. Leaves are fed to silkworm *(Boroceras cajani)* in southern Madagascar (Watt, 1908). In the Philippines, China, and India, pigeonpea plants are used to cultivate *(Laccifera lacca)* a scale insect, which produces lac.

The curative effects of various pigeonpea plant parts find place in folk and ayurvedic medicines in India, Indonesia, China, West Africa, Madagascar, and the West Indies—for healing wounds, destroying internal worms, and curing lung diseases (Morton, 1976). Prema and Kurup (1973) reported that pigeonpea intake decreased cholesterol in rats. Also Ekeke and Shade (1985) found that pigeonpea caused reversion of sickled cells in patients suffering from sickle cell anemia.

4.9 LOOKING AHEAD

Pigeonpea remains a less-domesticated plant, even after centuries of cultivation since it has retained its unique characteristics such as perenniality, nondeterminate growth, low harvest index, and photothermal sensitivity. However, multiple uses and its role in sustaining productivity makes pigeonpea a favorite crop of small land holders. In the last few decades, a significant progress has been made in domesticating the crop by developing short-duration and determinate types, but a large scope for further improvement still exists.

In recent years, pigeonpea production in India has recorded a significant growth rate, and it is attributed to the development of short-duration and medium-duration disease-resistant varieties. Since the demand for pigeonpea is ever increasing, the attention needs to be focused on increasing its yield potential. The exploitation of heterosis and restructuring of plant type are two possible ways of achieving breakthrough in yielding ability. To achieve this goal, a complementary approach is to knit these two and other important elements together. In pigeonpea, the information gap needs to be filled for significant yield increases at the genetic level. Restructuring plants is a difficult task, and significant input from physiologists is essential. In the subtropical environments where plants have sufficient biomass, the inefficient partitioning is the major yield-limiting factor. In this context, it is postulated that if the intra-plant competition for photosynthates is increased by inducing synchrony in fertilization and pod set in the entire plant, it may help in releasing the stored assimilates from stem, roots, and other plant parts, and it might lead to quick grain filling and increased yield. In the tropical environments and postrainy season pigeonpeas, where restricted biomass is the major production constraint, the hybrids are the answer because hybrids can produce about 25 to 30% additional biomass. The GMS hybrid technology in the past 25 years has conclusively demonstrated that the exploitation of hybrid vigor is feasible if the seed production difficulties are addressed adequately. The issues of developing high-yielding, CMS-based hybrids and their grower-friendly seed production technology also need further exploring. These include diversification and stability of cytoplasmic male sterility, breeding high-yielding diseases resistant ‘A’, ‘B’, and ‘R’ lines—and identification of heterotic cross combinations.

There have been debates in various international forums about the future of pigeonpea, because it has largely been treated as a regional crop, notwithstanding the multiple uses it offers. Among the factors contributing to its limited adoption outside India seem to be a lack of appreciable consumption due largely to different dietary habits, lack of adaptation into the cropping systems, and lack of appropriate appreciation of its diversified uses. However, with the recognition of its role in sustainability, development of new plant types amenable to mechanical harvest, resistance to major diseases, and extended adaptation from 30 to 45°N and S latitudes, it may be accepted as an alternative crop for diversification.

With the emphasis being placed on the intensification of agriculture, the crop will find increasing acceptance only if its yields can be improved both on per unit area and time basis. The development of short-duration cultivars and hybrids that can be commercially cultivated holds promise in this regard. The development of vegetable types has opened potential for encouraging rural enterprise and helping women obtain earning opportunities through canning and developing other marketable food items, such as noodles; fermented foods such as tempeh; and in sauces, as a substitute for soybean. The economic conditions in southeast Asia and frequent droughts are encouraging countries like Indonesia, China, and Myanmar to grow pigeonpea. The crop is being grown in Thailand for green manuring. In the U.S., there is increasing interest in growing pigeonpea crops for grazing during the lean season; the bushy dwarf types fit well into the production system likely to be followed there.

There are a number of other situations where pigeonpea can be successfully grown. Development of a pigeonpea simulation model can help in visualizing scenarios for assessing the profitability of the crop. This has been lacking so far, since such assessment required the actual conduct of trials at a considerable cost. The modeling tool can now be used for verification of potential, thus saving costs. However, a lot will still depend on how the crop will be consumed. In countries where people depend on meat as a source of protein, pigeonpea needs to be tried as animal fodder. Several studies have shown that it can be used for feeding poultry and pig. Other reports suggest using it for producing paper pulp, rearing lac insect, but definitive work needs to be undertaken in these areas. Some of these innovative uses will need to be explored if pigeonpea is to be made a truly global crop, which in turn is likely to attract more research investment for its improvement.

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CHAPTER 5
Cowpea [*Vigna unguiculata* (L.) Walp.]

B.B.Singh

5.1 INTRODUCTION

Cowpea, [*Vigna unguiculata* (L.) Walp.], also known as blackeye pea, southern pea, zipper pea, niebe, and lobia is one of the most important food legumes in the semi-arid tropics covering Asia, Africa, southern Europe, and Central and South America (Henriet et al., 1997; Mortimore et al., 1997; Singh et al., 1997a; van Ek et al., 1997). A drought tolerant and warm weather crop, cowpea is well adapted to the drier regions of the tropics, where other food legumes do not perform well. It also has the unique ability to fix atmospheric nitrogen through its nodules, and it grows well even in poor soils with more than 85% sand and with less than 0.2% organic matter and low levels of phosphorus (Kolawale et al., 2000; Sanginga et al., 2000). In addition, it is shade tolerant and, therefore, compatible as an intercrop with maize, millet, sorghum, sugarcane, and cotton as well as with several plantation crops (Singh and Emechebe, 1998), and thus it forms a valuable component of the traditional cropping systems. Coupled with these attributes, its quick growth and rapid ground cover checks soil erosion and in-situ decay of its roots, and nitrogen-rich residue improves soil fertility and structure, which together have made cowpea an important component of subsistence agriculture, particularly in the dry savannas of Sub-Saharan Africa (Carsky et al., 2001; Mortimore et al., 1997).

The important cowpea-growing countries are Nigeria, Niger Republic, Mali, Burkina Faso, Senegal, Ghana, Togo, Benin, Cameroon, and Chad, in Central and West Africa; Sudan, Somalia, Kenya, Malawi, Uganda, Tanzania, Zambia, Zimbabwe, Botswana, South Africa, and Mozambique, in East and Southern Africa; Bangladesh, China, India, Indonesia, Korea, Myanmar, Nepal, Philippines, Sri Lanka, and Thailand, in Asia; and Brazil, Cuba, Haiti, the U.S., and the West Indies, in North, Central, and South America. Reliable statistics are not available, but based on the FAO data (FAOSTAT-2003) and on correspondence with national programs, the estimated worldwide area under cowpea is more than 14 million ha, with more than 4.5 million t annual production. The available data on area, production, and average yield of cowpea in 11 important cowpeagrowing countries is presented in Table 5.1, which totals up to 11.3 million hectare and 3.6 million tons. These data indicate that a substantial part of the cowpea production comes from only a few countries. Nigeria is the largest producer and consumer of cowpea, with about 5 million ha area and about 2.4 million t produced annually. Niger Republic is the next

largest producer, with 3 million ha and more than 350,000 t produced. Northeast Brazil grows about 1.5 million ha of cowpea, with about 491,558 t produced, which provides food to about 25 million people. In the southern U.S., about 40,000 ha of cowpea is grown, with an estimated 45,000 t annual production of dry cowpea seed and a large amount of frozen green cowpeas. India is the largest cowpea producer in Asia, and together with Bangladesh, Indonesia, Myanmar, Nepal, Sri Lanka, Pakistan, Philippines, Thailand, and other far eastern countries, there may be more than 1.5 million ha under cowpea in Asia.

Cowpea is of major importance to the livelihoods of millions of people in less developed countries of the tropics. It is consumed in many forms. Young leaves, green pods, and green seeds

Table 5.1 Major Cowpea Growing Countries in the World (2003)

Country	Area Under Cowpea (Ha)	Production (t)	Yield (kg/ha)
Nigeria	5,050,100	2,389,000	417
Niger	3,800,000	350,000	171
Brazil	1,500,000	491,558	324
Mali	512,455	113,000	220
Tanzania	145,000	47,000	317
Myanmar	105,000	100,000	952
Uganda	64,000	64,000	1000
Haiti	55,000	38,500	700
U.S.	40,000	45,000	1000
Sri Lanka	15,000	12,130	808
South Africa	13,000	5,600	430
Total	11,299,555	3,674,788	324

Source: FAOSTAT and national reports.

are used as vegetables, and dry seeds are used in various food preparations (Nout, 1996; Nielsen et al., 1997). With 25% protein (on dry-weight basis) in its seeds and tender leaves (Bressani, 1985; Nielsen et al., 1997), cowpea is a major source of protein, minerals, and vitamins in the daily diets in Africa, and thus it positively influences the health of men, women and children. The bulk of the diet of the rural and urban poor in Africa consists of starchy food made from cassava, yam, plantain and banana, millet, sorghum, and maize. The addition of even a small amount of cowpea ensures a nutritional balance and enhances the protein quality by the synergistic effect of high protein and high lysine from cowpea and high methionine and high energy from the starchy foods. Trading fresh produce and processed cowpea foods and snacks provides rural and urban women opportunity for earning cash income.

Cowpea is equally important as nutritious fodder for livestock, particularly in the dry savannas of West Africa (Singh and Tarawali, 1997). Recently, Tarawali et al. (1997a, 1997b) reviewed the literature on the use of cowpea haulms as fodder in different parts of the world. In West Africa, the mature cowpea pods are harvested and the haulms are cut while still green and rolled into small bundles containing the leaves and vines. These bundles are stored on rooftops or on tree forks for use and for sale as “Harawa” (feed supplement) in the dry season, making cowpea haulms the key factor for crop-livestock systems (Singh and Tarawali, 1997). On the dry-weight basis, the price of cowpea haulms ranges between 50 and 80% of the grain price, and therefore, haulms constitute an

important source of income. Just like cowpea grains and leaves, the nutritive value of cowpea haulms is also very high. The crude protein content ranges from 13 to 17% in cowpea haulms, with high digestibility and low fiber (Tarawali et al., 1997b), and thus, cowpea fodder is a good protein supplement to cereal stalks for feeding livestock.

5.2 ORIGIN AND DISTRIBUTION

Major diversity in cowpea is found in Asia and Africa, but the precise origin of cowpea has been a matter of speculation and discussion for many years. Early observations showed that cowpeas in Asia were divers and morphologically different from those in Africa. Therefore, both Asia and Africa were thought to be independent centers of origin of cowpea. However, in the absence of wild cowpeas in Asia as possible progenitors, an Asian center of origin has recently been questioned. All the current evidence suggests that cowpea originated in Southern Africa, although it is difficult to ascertain where in Africa the crop was first domesticated. Several centers of domestication have been suggested, such as Ethiopia (Vavilov, 1951; Sauer, 1952; and Steele, 1976), Central Africa (Piper, 1913), South Africa (Zhukovaskii, 1962), and West Africa (Faris, 1965; Rawal, 1975; Lush and Evans, 1981).

Based on the distribution of diverse wild cowpeas in Eastern Africa, stretching from Ethiopia to Southern Africa, the working group meeting of the International Board for Plant Genetic Resources on *Vigna,* held in New Delhi (IBPGR, 1981), recommended as a priority the collection of both wild and cultivated forms of cowpea in Southern Africa, Zimbabwe, Transvaal, and Natal. Baudoin and Maréchal (1985) considered East and Southern Africa to be the primary regions of diversity, and West and Central Africa to be the secondary centers of diversity. They also proposed Asia to be the third center of diversity. Recent investigations by the International Institute of Tropical Agriculture (IITA), in collaboration with Instituto del Germoplasmo (CNR) Bari, Italy, strongly indicate that the region encompassing Namibia, Botswana, Zambia, Zimbabwe, Mozambique, Swaziland, and South Africa has the highest genetic diversity with respect to primitive wild forms of cowpea (Padulosi et al., 1990, 1991; Padulosi, 1993). Some very primitive species were observed in the Transvaal, Cape Town, and Swaziland. Based on this, Padulosi and Ng (1997) suggested that Southern Africa may be the origin of cowpea, and from there primitive forms moved to other parts in Southern and Eastern Africa, and from there to Asia and West Africa. Since cowpea was known in India before Christ, and it has a Sanskrit name in an early treatise dating back to 150 BC (Steele and Mehra, 1980), cowpea must have moved from East Africa to Asia more than 2000 years ago, when human selection led to modified forms of cowpea different from that in Africa. Ng and Maréchal (1985) suggested that cowpea probably moved from Eastern Africa to India before 150 BC, to West Asia and Europe about 300 BC, and to the Americas in 1500 AD. Since Western Asia and Europe do not have desired climatic conditions for cowpea, not as much variability and selection occurred as in South Asia and South East Asia, where small seeded and vegetable cowpeas were selected. The wild cowpeas with very small seeds were probably distributed by birds in East and West Africa long before the Christian Era, and therefore, there exist great diversity and secondary wild forms there. Selections for larger seeds and better growth habits from natural variants in wild

cowpeas by humans must have led to diverse cultigroups and their domestication in Asia and in Africa (Ng, 1995). Using chloroplast DNA polymorphism, Vaillancourt and Weeden (1992) suggested Nigeria to be the center of domestication in West Africa.

5.3 TAXONOMY

Cowpea is a Dicotyledonea belonging to the order Fabaceae, subfamily Faboideae (Syn. Papillionoideae), tribe Phaseoleae, subtribe Phaseolinae, genus *Vigna*, and section *Catiang* (Verdcourt, 1970; Maréchal et al., 1978). *Vigna* is a pantropical and highly variable genus with several species, the number varying from 84 to 184 (Phillips, 1951; Faris, 1965; Verdcourt, 1970; Steele, 1976; Maréchal et al., 1978; Summerfield and Roberts, 1985). The genus *Vigna* has been subdivided into seven subgenera: *Vigna, Sigmoidotropis, Plectotropis, Macrophynca, Ceratotropis, Haydonia,* and *Lasiocarpa* (Maréchal et al., 1978). The subgenus *Vigna* has been futher subdivided into six sections: *Vigna, Comosae, Macrodontae, Reticulatae, Liebrechtsia,* and *Catiang*. Cowpea belongs to section *Catiang,* which, according to Verdcourt (1970), consisted of five species, *V. unguiculata, V. pubescence, V. augustifoliolata, V. tenuis,* and *V. nervosa*. However, Maréchal et al. (1978) reduced these to only two species, *V. unguiculata* and *V. nervosa,* and classified the others into subspecies of *V. unguiculata*.

Vigna unguiculata has been further subdivided (see Table 5.2). The cultivated cowpea was earlier divided into four subspecies (Verdcourt, 1970), but these are now accepted to be four cultigroups within the subspecies *unguiculata*. The cultigroups are *unguiculata, biflora* (or *cylindrica*), *sesquipedalis,* and *textilis* (Westphal, 1974; Maréchal et al., 1978; Ng and Maréchal, 1985). A brief description of the four cultigroups under subspecies *unguiculata* is presented below:

Table 5.2 Classification and Nomenclature of *Vigna unguiculata* Species Complex

Maréchal et al. (1978)	Pienaar (1992)	Pasquet (1993a)	Padulosi (1993)
V. unguiculata	*V. unguiculata*	*V. unguiculata*	*V. unguiculata*
	Cultivated Forms		
ssp. *unguiculata*	ssp. *unguiculata*	ssp. *unguiculata*	ssp. *unguiculata*
	Cultigroups: unguiculata, biflora/cylindrica, sequipedalis and textilis		
	Wild Forms		
ssp. *Dekindtiana*	ssp. *Dekindtiana*	ssp. *dekindtiana*	ssp. *dekindtiana*
var. *dekindtiana*	var. *dekindtiana*	var. *dekindtiana*	var. *dekindtiana*
	var. *huliensis*		var. *huliensis*
			var. *congolensis*
			var. *grandiflora*
var. *mensensis*	ssp. *mensensis*	ssp. *Letouzeyi*	var. *ciliolata*

		ssp. *burundiensis*	
		ssp. *baoulensis*	
var. *protracta*	ssp. *mensensis*	ssp. *stenophylla*	ssp. *Protracta*
			var. *protracta*
			var. *kgalagadiensis*
			var. *rhomboidea*
var. *pubescens*	ssp. *protracta*	ssp. pubsescens	ssp. *pubescens*
Ssp. *Stenophylla*	ssp. *Stenophylla*	ssp. Stenophylla	ssp. *stenophylla*
Ssp. *tenuis*	ssp. *Tenuis*	ssp. tenuis	ssp. *tenuis*
	var. *tenuis*		var. *tenuis*
	var. *ovata*		var. *oblonga*
			var. *parviflora*

1. Cultigroup *unguiculata:* pods 10 to 30 cm long, pendant, seeds 5 to 12 mm long, originally from Africa and now popular world over
2. Cultigroup biflora or *cylindrica:* pods 7.5 to 13 mm long, usually erect, seeds 5 to 6 mm long, from India, mostly grown for fodder and seeds in Asia and used as pulse
3. Cultigroup *sequipedalis:* very long and succulent pods 30 to 60 cm in length, also called yardlong bean and asparagus bean, from India, southeast Asia, and China, with seeds usually 8 to 12 mm long, mostly grown in Asia as a vegetable
4. Cultigroup *textiles:* long fibrous peduncles 50 to 80 cm long, mostly grown in northern Nigeria for fiber

The classification and nomenclature of the immediate wild progenitors of cultivated cowpeas was first proposed by Verdcourt (1970) and subsequently revised by Maréchal et al. (1978), Piennar and van Wyk (1992), Pasquet (1993a), and Padulosi (1993). An extensive work on characterization of more than 400 wild *V. unguiculata* accessions was conducted at IITA (Ng and Padulosi, 1991; Padulosi, 1993). This work, coupled with surveys of live materials in the field and specimens in major herbaria in Europe and Africa, as well as cytological studies, have led to the description of new species and a change of nomenclature of some species (Padulosi, 1993; Ng, 1995). For clarity, the synonyms of the various wild *V. unguiculata* species and their classification system proposed by different researchers are listed in Table 5.2. In the classification system proposed by Padulosi (1993), the three subspecies *dekindtiana, tenuis,* and *stenophylla* proposed by Maréchal et al. (1978) were retained. However, var. *protracta* and var. *pubescens* were raised to the level of two distinct subspecies because of their very distinctive hairy characteristics in pods and other plant parts, morphology of their flowers, pollen, grains, and leaves, as well as their root systems. Within the subspecies *protracta,* three varieties, namely var. protracta, var. *rhomboidea,* and var. *kgalagadiensis,* were distinguished. Similarly, the three varieties *tenuis, oblonga,* and *parviflora* were recognized within the subspecies *tenuis,* while four new varieties, var.

huillensis, var. *congolensis*, var. *ciliolate*, and var. *grandiflora*, have also been proposed and added to the subspecies *dekindtiana*.

The discussion on the taxonomic relations within *V. unguiculata* continues. Ng (1995) proposed to reinstate var. rhomboidea to a species ranking in its own right, because of its strong incompatibility with other taxa within *V. unguiculata*. Pasquet (1993a) proposed that the name subspecies *unguiculata* var. spontanea be used to describe all the weedy forms and the intermediates between truly wild var. dekindtiana and cultivated cowpea. The subspecies *burundiensis* (Pasquet, 1993a) is a variant of var. ciliolate. It is found in mid-altitudes in Zaire, Burundi, Kenya, and Uganda.

Great variability in plant morphology, including leaf type, growth habit, maturity, pod type, seed type, and color, has been observed in wild cowpeas (Vaillancourt and Weeden, 1992; Vaillancourt et al., 1993; Panella et al., 1993; Pasquet, 1993b). The subsp. *pubescens* and *protracta* are pubescent, with their stems, leaves, and pods covered with hairs. However, the hairs of subsp. *pubescence* are silky, straight, soft, and appressed to the surface of the stems and pods. On the other hand, the hair types of subsp. *protracta* are bristly, erect, straight, and harshly stiff.

5.4 GENE POOL

A good knowledge of crop taxonomy contributes to an efficient use of germplasm for hybridization in the breeding programs. In addition, the results of hybridizations and cross compatibilities provide the basis for improving plant classification. This approach has permitted grouping of the germplasm available for hybridization into primary, secondary, or tertiary gene pools (Harlan and de Wet, 1971). The primary gene pool includes both the cultivated and the wild forms, which are easily hybridized. The secondary and the tertiary gene pools comprise all the species among which gene flow is possible through interspecific hybridization but with increasing degrees of difficulty. The boundary between secondary and tertiary gene pools may not always be well defined. In general, when the cross is successful but the F_1 hybrid is less fertile or presents moderate structural heterology, the donor species will then be considered to belong to the secondary gene pool. Thus, the secondary gene pool comprises other species that are relatives of the crop and are suitable for interspecific hybridization. When a cross between a cultivated and a donor species is possible with difficulty, and the F_1 hybrid is sterile or presents major meiotic irregularities because of marked structural differentiation between the two genomes, then the donor species is considered to belong to the tertiary gene pool of the cultivated species. Thus, the tertiary gene pool involves still greater barriers to hybridization compared to the secondary gene pool, embracing species that display either unviable or sterile hybrids with the cultivated plant and do not permit gene flow by conventional methods of introgression.

Using these criteria, the primary gene pool of cowpea comprises four cultigroups of the subspecies *unguiculata*, and most of the varieties of subspecies *dekindtiana*, *stenophylla*, and *tenuis* as indicated in Table 5.2. However, some of the wild subspecies, like *pubescence*, are not freely crossable but need embryo rescue and show some level of pollen sterility (Fatokun and Singh, 1987). Therefore, some of the subspecies of *V. unguiculata* may constitute a secondary gene pool. Recent attempts to cross *V. vexillata*

and *V. radiata* with *V. unguiculata* (Barone et al., 1992; Gomathinayagam et al., 1998; Fatokun, 2002) indicate the possibility of reaching to the tertiary gene pool. Barone et al. (1992) observed occasional fertilization between *V. vexillata* and *V. unguiculata*, but the fertilized embryos degenerated within a few days. Gomathinayagam et al. (1998) reported successful cross between *V. vexillata* and *V. unguiculata*. However, the resulting F_1 hybrid and backcross plants looked more like *V. vexillata* plants, but these were not followed systematically to ascertain the veracity of the cross. Fatokun (2002) reported various levels of pollen tube elongation in crosses between *V. vexillata* and *V. unguiculata* and tried to rescue embryos, but no success was achieved. Tyagi and Chawala (1999) reported successful cross between *V. radiata* and *V. unguiculata* using *in vitro* culture technique. About 10% of the total embryos cultured resulted in plantlet formation, but they did not report further growth and culture of these plantlets to confirm the true nature of the cross. *Vigna vexillata* belongs to the subgenus *Plectotropis*, and *V. radiata* belongs to subgenus *Ceratotropis* within the genus *Vigna*. Using isozyme and RFLP analysis, Fatokun et al. (1993) and Sonnante et al. (1997) found *V. vexillata* to be closer to *V. unguiculata* than other species. There is a need to continue efforts to cross these and other species with *V. unguiculata* and determine if these constitute a tertiary gene pool for cowpea.

5.5 GERMPLASM RESOURCES

The significance of cowpea germplasm collection, evaluation, preservation, and use for improving cowpea cultigens has been reviewed (Steele et al., 1985; Ng, 1987a). Prior to 1967, a few national agricultural research programs, such as Nigeria, Senegal, Tanzania, and Uganda in Africa; India in Asia; and the U.S. in the Americas, had some level of cowpea improvement program, and they were maintaining some collection of cowpea germplasm. However, with the establishment of the International Institute of Tropical Agriculture (IITA) in 1967 and its global mandate for the improvement of cowpea, IITA has made a collection of cowpea germplasm exceeding 15,100 accessions of cultivated varieties drawn from more than 100 countries and 560 accessions of wild cowpeas (Table 5.3). The germplsm lines maintained at IITA have been numbered as TVv (Tropical *V. unguiculata*, i.e., TVu-1, TVu-2, etc.). These have been characterized and evaluated for desirable traits and are being preserved and used in the breeding program at IITA, as well as in national breeding programs (Ng and Padulosi, 1991). In the collection, wide variability in cowpea genotypes has been observed with respect to many traits. These traits are plant pigmentation, plant type, plant height, leaf type, growth habit, photosensitivity and maturity, nitrogen fixation, fodder quality, pod traits, seed traits, and grain quality. The economically useful traits such as heat and drought tolerances; root architecture; resistance to major bacterial, fungal, and viral diseases; resistance to root knot nematodes; resistance to aphid, bruchid, and thrips; and resistance to parasitic weeds such as *Striga gesnerioides* and *Alectra vogelii* are available in secondary and tertiary gene pools.

These large and diverse cowpea germplasm collections are available to all the cowpea researchers around the world, especially in Africa, to exploit the valuable genes to improve cowpea cultivars.

5.5.1 Sources of Resistance to Insect Pests

Through intensive screening of the existing collections at IITA, many sources of resistance to insect pests like cowpea aphid *(Aphis craccivora),* leafhoppers (*Empoasca signata* and *E. dolichi*), legume bud thrips *(Megalurothrips sjostedti),* pod borers *(Maruca vitrata),* pod-sucking bugs *(Clavigralla sp.),* and bruchids *(Callosobruchus maculates)* have been identified (Singh, 1977, 1980; IITA, 1978, 1983; Singh and Allen, 1979; Singh el al., 1983). These sources are summarized in Table 5.4. Among the lines identified, the level of resistance is high only for aphid and bruchid, moderate for thrips, but low for Maruca and pod bugs. Using TVu-410, TVu-801, and TVu-3000 for aphid resistance, TVu-1509 for thrips resistance, and TVu-2027 for bruchid resistance, a number of improved varieties have been developed with combined resistance to these insects and several diseases. Some of the varieties that have already become popular in Nigeria are IT89KD-288, IT90K-277–2, IT93K-452–1, and IT97K-499–35.

Table 5.3 Cowpea Germplasm Accessions at IITA Collected from Different Countries

Country	No. Accessions	Country	No. Accessions
Central and West Africa		Asia (continued)	
Burkina Faso	222	Israel	8
Cameroon	600	Iran	29
Central Africa Republic	183	Japan	2
Chad	268	Laos	1
Gambia	4	Nepal	3
Ghana	282	Pakistan	7
Guinea	2	Papua New Guinea	12
Côte d'lvoire	134	Philippines	114
Liberia	9	Sri Lanka	2
Mali	293	Syria	8
Niger	976	Thailand	1
Niger (+ IITA)	3,221	Turkey	47
Republic of Benin	331	USSR	42
Senegal	290	North and South America	
Sierra Leone	13	Argentina	1
Togo	103	Brazil	171
Zarïe	15	Canada	182
East and Southern Africa		Colombia	2

Angola	1	Cuba	1
Botswana	457	El Salvador	1
Congo	47	Guatemala	11
Ethiopia	7	Honduras	1
Madagascar	34	Jamaica	1
Malawi	494	Mexico	23
Lesotho	42	Nicaragua	2
Swaziland	19	Paraguay	12
Somalia	3	Peru	3
Tanzania	495	Suriname	14
Uganda	71	USA	828
Zambia	587	Venezuela	3
Zimbabwe	158	Europe	
North Africa		UK	282
Algeria	1	Hungary	36
Egypt	347	Portugal	5
Asia		Italy	93
Afghanistan	65	Australia	23
Bangladesh	1	Unknown	610
China	35	Ex-mix	630
India	2,075	Wild Cowpeas	560
Indonesia	4	**Total**	**15,660**

Table 5.4 Cowpea Germplasm Lines Resistant to Various Insect Pests

Pest	Sources of Resistance (IITA's TVu No.)
Aphid	35, 57, 134, 157, 191, 200, 310, 308, 410, 801, 2000, 2657, 2755, 2845, 2896, 3273, 3346, 3433, 9836, 9914, 9929, 9930, 9944
Leaf hoppers	50, 59, 123, 305, 418, 662, 1037, 1045, 1190
Legume bud thrips	1509, 2870 (all moderate resistance)
Legume pod borers	946, 13271, VITA-5 (all low level of resistance)
Cydia ptychora	4579, 4328 (moderate resistance)

Pod-sucking bugs	1977, 7274
Bruchids	2027, 11952, 11953

Table 5.5 Cowpea Germplasm Lines Resistant to Major Diseases*

Disease	Locality of Screening	Resistant Germplasm Accessions (IITA's TVu No)
Brown blotch	Nigeria	201, 1977
Scab	Nigeria	843, 1404, 1433, 1977
Bacterial blight	Nigeria	347, 410, 456, 483–2, 726, 745, 1190, 1977
Septoria	Nigeria	456, 483, 486, 726, 853, 1433, 1583, 2455, 1977
Fusarium wilt	Nigeria	109, 347, 984, 1000, 1016
Fusarium root rot	Puerto Rico	202, 231, 243, 266, 274, 320, 316, 393, 408, 1563
Phakopsora rust	Nigeria and Uganda	612, 1258, 1962, 2455, 4540
Web blight	Nigeria	317, 2483, 4539 (all are moderately resistant)
Synchytrium false rust	Uganda	43, 222, 612, 4535, 4537, 4569, 6666
Target spot	Nigeria	1190
Root knot nematodes	Nigeria	264, 401, 857, 1560
Bacterial blight+scab+		
Septoria+brown blotch	Nigeria	1977
Scab+Septoria	Nigeria	853, 1433
Bacterial blight+Septoria	Nigeria	456, 4832, 726
Anthracnose+bacterial pustule	Nigeria	201, 347, 408, 410, 537, 697, 746,
Cercospora leaf spot+rust +		1190, 1283, 2430, 3415, 310, 345,
Cowpea yellow mosaic virus		393, 645, 990, 1452, 1980, 2755, 3565
Major Viruses	Nigeria	201,410, 1190
Striga and Alectra	Nigeria	9238, 11788, 12415, 12432, 12470, B301

* Ng and Padulosi (1991) and Singh and Emechebe (1997).

5.5.2 Sources of Resistance to Disease and Parasitic Plants

The cowpea germplasm has also been screened for sources of resistance to major diseases (Williams, 1975, 1977a, 1977b; Ladipo and Allen, 1979; IITA, 1978; Singh and Allen, 1979; Singh et al., 1983), and these resistant sources are listed in Table 5.5. During the field evaluation of cowpea germplasm collection, four lines, TVu-201, TVu-1190, TVu-1977, and TVu-4577, were found to be resistant to many diseases and had very high yield potential. These were named as VITA-1, VITA-3, VITA-4, and VITA-5 (Vigna IITA-1, 3, 4, 5), respectively, and released in many countries (Singh and Ntare, 1985). These VITA lines have also been extensively used as parents in cowpea breeding programs.

5.6 CYTOGENETICS AND INTERSPECIFIC HYBRIDIZATION

Only limited studies on cowpea cytogenetics have been conducted. A few researchers have counted chromosomes in *V. unguiculata* and unanimously observed the 2n=2x=22 chromosomes (Faris, 1964; Frahm-Leliveld, 1965; Yarnell, 1965). A detailed study of the pachytene chromosomes by Mukherjee (1968) revealed that the 11 bivalent complement in cowpea consisted of 1 short (19 µm), 7 medium (26–36 µm), and 3 long (41–45 µm) chromosomes. They also observed that chromomeres were not distributed uniformly along the arms.

A few recent studies using newer techniques have given detailed descriptions of the cowpea karyotype. Barone and Saccardo (1990) used pachytene bivalents to develop their karyotype. Pignone et al. (1990) developed a banded karyotype by using mitotic prometaphase chromosomes. Saccardo et al. (1992) used conventional techniques and an automatic image analysis system in their work with pachytene and mitotic prometaphase and metaphase chromosomes. Galasso et al. (1997) used Cbanding and fluorescent in-situ hybridization (FISH) techniques and found that most of the heterochromatin had a cetromeric distribution and all the chromosomes showed centromeric heterochromatin blocks. Venora and Padulosi (1997) carried out a karyotypic analysis of mitotic chromosomes of 11 wild subspecies of *V. unguiculata* and found a low degree of karyological variability. This fits with free crossing between the subspecies within *V. unguiculata.* However, Fatokun and Singh (1987) could obtain successful hybrids between *V. pubescence* and *V. unguiculata* only by embryo rescue technique, and they observed only about 32% viable pollen in the F_1 plants. Cytological investigations of F_1 plants showed some meiotic abnormalities in the pollen mother cells. These abnormalities included a few univalents and quadrivalents, suggesting some structural differentiation in the chromosomes.

Barone and Ng (1990) could not obtain an interspecific cross between *V. vexillata* and *V. unguiculata,* due to slow pollen tube growth, infrequent fertilization, and collapse of ovules 5 to 8 days after fertilization. Evans (1976) failed in all efforts to cross *V. unguiculata* with *V. vexillata,* and all attempts by Fatokun (1991) to cross *V. vexillata* with various cultivated and noncultivated cowpeas were unsuccessful. Gomathinayagam et al. (1998) reported a successful cross between *V. vexillata* and *V. unguiculata* using embryo culture. They obtained 13 hybrid plants that showed intermediate morphological

traits between the parents for leaf shape, pod color, and seed coat color. However, the stem and leaf types and pod hairiness of hybrid plants were like those of *V. vexillata*, the maternal parent. Electrophoresis studies of the hybrid plants for peroxidase and esterase and cytological studies confirmed that they were true hybrids. However, the authors did not follow through the progenies to ensure agronomic confirmation of the cross. The same cross has not been successful at IITA (Fatokun, 1997). An interesting observation by both researchers was that the pollinated buds stayed longer (4 to 7 days) and showed pod formation only when *V. vexillata* was the female, but dropped within 24 h when *V. unguiculata* was the female.

A report of an attempted wide cross between cowpea *(V. unguiculata)* and bambara groundnut *(V. subterranea)* was published by Begemann et al. (1997). The cowpea lines TVu 13677 was crossed as a female parent with bambara groundnut variety TVsu-501 as a male. The crossed flowers produced an abnormally short pod (about 1 cm) with only one seed. The F_1 seed gave rise to a plant that had a longer growth period (80 days) than the female parent TVu 13677 (60 days). Similarly, Tyagi and Chawala (1999) reported a successful cross between *V. radiata* and *V. unguiculata* using *in vitro* culture method. However, none of the two authors followed up with the hybrid populations to confirm whether these plants were real hybrids.

The cowpea has not been successfully hybridized with any other *Vigna* or *Phaseolus* species. Singh et al. (1964) were unable to cross *V. unguiculata* with *V. umbellate, V. mungo, V. radiata, V. aconitifolia,* and *V. angularis.* Attempts by Ballon and York (1959) to obtain intergeneric crosses between *V. unguiculata* and *P. coccineus* and *P. vulgaris* were also unsuccessful.

Induced tetraploid forms of *V. unguiculata* have been studied by Sen and Hari (1956) and Sen and Bhowal (1960), who reported marked cultivar differences in morphologic traits, pollen sterility, fruit setting, and meiotic irregularities including the formation of quadrivalents, trivalents, and univalents and the presence of laggards and unequal distribution of chromosomes in the anaphase stage. They concluded that pollen sterility was the factor most limiting to the development of commercially useful tetraploid cultivars.

Ghosh (1978) induced cowpea tetraploids and noted abnormalities with many plant traits, e.g., poor germination of seeds, poor seedling growth and plant survival, leaflet shape and color, stomata size, time and duration of flowering, flower size, pollen grain viability, number of shriveled seed per pod, and seed size.

5.7 COWPEA GENETICS

Among the cultivated crops, cowpea is the most variable species with extreme genotypes and a very short life cycle (55 days). Therefore, it is an ideal species for genetic studies. However, being a minor crop, and of African origin, it has not received much attention from geneticists. Cowpea genetic research began in the early 1900s (Spillman, 1911, 1913; Harland, 1919a,b, 1920), and after a long gap, it began again in the early 1960s (Saunders, 1960a,b; Sen and Bhowal, 1961) and continued thereafter. These studies have elucidated inheritance of many traits and given suitable

Table 5.6 List of Gene Symbols for Important Traits in Cowpea

Trait	Gene Symbol	Reference
Vining growth	Vi-1, Vi-2,Vi-3	Brittingham (1950), Norton (1961), Singh and Jindla (1971)
Hastate leaf (narrow)	Ha (incomplete dominance)	Ojomo (1977)
Early flowering	Ef-1, Ef-2	Ojomo (1971)
Male sterility	ms1, ms2, pms	Sen and Bhowal (1962), Rachie et al. (1975), Singh and Adu-Dapaah (1998)
Photosensitivity	Ps	Ishiyaku and Singh (2001)
Smooth seed texture	Rt_1, Rt_2	Singh and Ishiyaku (2000)
Resistance to:		
Drought stress	Rds_1, Rds_2	Mai-Kodomi et al. (1999b)
Bacterial pustule	Bptl-$_1$, Bpl-$_2$	Patel (1982)
Bacterial canker	BC-$_1$, Bc-$_2$	Singh and Patel (1977), Fery (1980)
Verticillium wilt	Vw	Moore (1974)
Cercospora leafspot	Cls-$_1$, Cls-$_2$	Fery and Dukes (1977)
Striga gesnerioides	Rsg-$_1$, Rsg-$_2$, Rsg-$_3$	Singh and Emechebe (1990), Atokple et al. (1995)
Alectra vogelii	Rav-$_1$, Rav-$_2$	Singh et al. (1993)
Septoria leafspot	RSV-$_1$, Rsv-$_2$	Abadassi et al. (1987)
Uromyces rust	UV-$_1$, Uv-$_2$	Chen and Heath (1993)
Root knot nematode	Rk, rk	Fery and Dukes (1980, 1982)
Black eye mosaic	blc	Walker and Chambliss (1981)
Southern bean mosaic	Sbm	Brantley and Kuhn (1970)
Tobacco ring spot	Tr	Dezeeuw and Ballard (1959)
Cowpea severe mosaic	ims	Jimenez et al. (1989)
Aphid	Rac	Bata et al. (1987)
Bruchid	rcm-$_1$, rcm-$_2$	Adjadi et al. (1985)

gene symbols. A detailed review of cowpea genetics was first published in 1980 (Fery, 1980) and subsequent supplements were published in 1985 (Fery, 1985), in 1997 (Fery and Singh, 1997), and in 2002 (Singh, 2002).

A total of 207 genes have been identified. These genes control plant pigmentation; plant type; plant height; leaf type; growth habit; photosensitivity and maturity; nitrogen fixation; fodder quality; heat and drought tolerances; root architecture; resistance to major bacterial, fungal, and viral diseases; reistance to root knot nematodes; resistance to aphid, bruchid, and thrips; resistance to parasitic weeds such as *S. gesnerioides* and *A. vogelii;* pod traits; seed traits; and grain quality. Quantitative genetic studies on heritability of yield and yield components and protein content and heterosis for important traits and improved breeding methods have facilitated selection of parents and increased efficiency in breeding programs. Table 5.6 lists some of the genes controlling important traits.

5.7.1 Genetics of Plant Pigmentation

Because of the great diversity in pigmentation of cowpea stem, leaf, flower, peduncle, petiole, and pod, this trait has been studied by a large number of researchers from 1919 to the present. However, since most of the studies involved pigmentation of one or the other plant parts at a time, there seems to be several gene symbols assigned for the same trait or similar traits. A close examination of gene symbols assigned for pigmentation of different plant parts by previous workers clearly indicates the overlap and confusion. For example, seven gene symbols (*Pp-1, Pp-2, pg, Pb, Pbr, Pu,* and *X*) have been assigned to plant pigmentation covering the plant, petiole base, branch base, stem-pod-petiole, and the vegetative parts, which obviously have overlaps. Similar overlaps

Table 5.7 Pigmentation of Different Parts in Selected Cowpea Varieties

	Pigmentation in Various Plant Parts										
						Flower					
Genetic Type	**Stem**	**Jts**	**Pet**	**Ped**	**Clx**	**s**	**w**	**k**	**Pd**	**Pdt**	**Seed Color**
TVx 3236-OC-1	–	–	–	–	–	–	–	–	–	–	—
IT87D-941–1	–	–	–	–	–	–	+	–	–	–	brown rough
TVx 3236-OC-2	–	–	–	–	–	–	+	–	–	–	brown rough
IT98K-628–2	–	+	–	–	+	–	–	–	+	+	white rough
IT90K-277–2	–	+	–	–	–	–	–	–	–	–	white rough
IT98K-598	–	+	–	–	–	+[2]	+[2]	+[2]	–	–	brown smooth
IT97K-1101–5	+	+[2]	+[2]	+[2]	+[2]	+[2]	+[2]	+[2]	+[2]	+[2]	black smooth
Kamboinse local	+[2]	+[2]	+[2]	+[2]	+[2]	b	–	–	+[2]	+[2]	white rough

IT86D-719	+	+	–	+	+[2]	–	–	–	+2	+[2]	white rough
IT95K-1491	–	+	–	–	+	+	+	–	+	+	white smooth

Note: Jts=joints, Pet=petiole, Ped=peduncle, Clx=calyx, Fl=flower, Pd=pod, Pdt=pod tip, s=standard, w=wing, k=keel, b=a purple dash at the back of the standard petal.

and confusion about the gene symbols are evident for flower color, calyx color, and pod color. None of the reports has endeavored to study plant pigmentation on a comprehensive basis explaining the relationship between pigmentation in different plant parts. Even the recent studies have not clarified the situation. Joshi et al. (1994) reported that P_1 is a pleiotropic gene for pigmentation in axil, calyx, corolla, pod tip, and seed, with localized genes conditioning coloration on individual parts. Uguru (1995) showed that petal color is controlled by one allelic pair, *WW*, while pod controls petal color and shoot colors appear to be determined pleiotropically by two allelic pairs, P_rP_r and G_rG_r Calyx color was reported to be controlled by three duplicate genes, and standard petal color is controlled by a single dominant gene. Biradar et al. (1997) reported three genes for calyx color, three genes for seed coat color, four genes for pod tip pigmentation, and four genes for flower color, with some genes showing pleiotropic effects. Venugopal (1998) observed that one to five pairs of genes were involved in the inheritance of plant pigmentation in cowpea. Sangwan and Lodhi (1998) studied the inheritance of flower color and pod color. They observed that purple flower color is dominant over white flower, and black pod color is partially dominant over white pod color, with monogenic inheritance for both traits.

The confusion about the genetics of plant pigmentation arises because most of the published reports do not give specific details of the pigmentation pattern and pigmented parts. For example, purple flower color does not mean much because pigmentation in cowpea flower may be restricted only to standard, wing, or keel petals or a combination of two or all three parts. A close examination (by the author) of several cowpea varieties has revealed very interesting and contrasting combinations of plant pigmentation (Table 5.7). It has also been observed (by the author) that all the cowpea varieties with brown rough seed have no pigmentation on any plant part except for a faint purple tinge on the inner margins of the standard petal. Also, all the cowpea varieties with white rough seed have purple pigmentation on the joints (bases of the branch, peduncle, petiole, and leaflets) that are always inherited as one gene. However, the pigmentation of whole stem, petiole, peduncle, and pod are independent of the pigmentation on the joints. It has also been observed that whenever the calyx is pigmented, the pod tips are also pigmented, and this is independent of other pigmentation. Another interesting pigmentation pattern is present in the cowpea variety Kamboinse local. It has dark purple pigmentation on stem, petiole, peduncle, joints, calyx, and pod, but the flowers are completely white except for a purple dash in the back of the standard. The cowpea varieties listed in Table 5.7 represent a good set of differentials for different pigmentation patterns, and these are being used in planned genetic studies (by the author) to elucidate inheritance pattern and interaction of any specific plant pigmentations.

5.7.2 Genetics of Disease Resistance

Inheritance of resistance to all the major cowpea diseases has been reported and reviewed by Fery (1980, 1985). This includes bacterial diseases such as pustule and bacterial blight; and fungal diseases like anthracnose, cercospora leaf spot, charcoal rot, Fusarium wilt, powdery mildew, cowpea rust, stem rot, target leaf spot, and Verticillium wilt. The virus diseases include bean yellow mosaic, blackeye cowpea mosaic, southern bean mosaic, aphid borne mosaic, cowpea mottle, cucumber mosaic, and tobacco ring spot, as well as root knot nematodes. The inheritance of resistance to brown blotch, Septoria leaf spot, Scab, phytophthora stem rot, and cowpea rust, virus diseases, and Striga and Alectra parasitic weeds were reviewed by Fery and Singh (1997). The recent reports on genetics of cowpea diseases were reviewed by Singh (2002). Vale and Lema (1995) studied the inheritance of resistance to cowpea severe mosaic comovirus (CpSMV) using cowpea variety Macaibo as the resistant parent and Pitiuba as the susceptible parent. The F_1 plants were uniformly susceptible and F_2 segregated into 3 susceptible to 1 resistant indicating involvement of a single recessive gene pair for resistance. The authors have mentioned that Macaibo is immune to CpSMV. Arshad et al. (1998) studied the inheritance of resistance to blackeye cowpea mosaic (BlCMV) in 6 cowpea varieties viz. IT86F-2089–5, IT86D-880, IT90K-76, IT86D-1010, IT86F-2065–5, and PB1CP3. The segregation pattern in F_1, F_2 and backcross populations suggested that the single recessive gene pair in each cowpea line controls the resistance to BlCMV. They designated *'bcm'* as the gene symbol.

Ryerson and Heath (1996) studied the inheritance of resistance to rust *(Uromyces vignae)* in cowpea cultivar 'Calico Crowder'. The segregation pattern in F_2 generation, and subsequent progeny, suggested the presence of multiple genes and the presence of dominant and recessive resistance components. Rangaiah (1997) also reported the inheritance of rust resistance in cowpea in 8 F_2 populations. He observed that a minimum of two genes control resistance to rust in cowpea. Nakawuka and Adipala (1997) screened 75 cowpea lines against scab, of which 10 were resistant. These lines were used to study the genetics of resistance to scab by Tumwegamire et al. (1998) using a half-diallel cross set. Broad sense heritability for foliar resistance was 93.8%, and for pod resistance it was 97 and 84.5%, respectively. This indicates major gene inheritance that had earlier been reported by Abadassi et al. (1987) in Tvx 3236.

Roberts et al. (1996) identified IT84S-2049 cowpea line from IITA to be completely resistant to diverse populations of the root knot nematodes, *Meloidogyne incognita* and *M. javanica*. The resistance in this variety was effective against nematode isolates that are virulent to the resistance gene 'Rk' present in commercial cultivars in California, like CB5 and CB46. Systematic genetic studies indicated that the resistance in IT84S-2049 was conferred by a single dominant gene, which was either allelic to the 'Rk' gene—or a different gene very closely lined to 'Rk'. Therefore, the symbol 'Rk2' was proposed to designate this new resistance factor. Rodriguez et al. (1996) artificially screened nine cowpea varieties for resistance to the root knot nematode *(M. incognita)*. They observed that IITA-3, Habana 82, Incarita-1, IT86D-364, IT87D-1463–8, Vinales 144, P902, and IITA-7 were highly resistant, whereas the local variety 'Cancharro' was highly susceptible.

5.7.3 Genetics of Insect Resistance

Inheritance of some important insect pests of cowpea has been studied and reported. Resistance to curculio was found to be quantitively inherited (Youngblood and Chambliss, 1993), but inheritance of cowpea aphid *(Aphis craccivora)*, is controlled by a single dominant gene (Bata et al., 1987), thrips resistance by two recessive genes (IITA, 1983), and bruchid resistance also by two recessive genes (Adjadi et al., 1985). Only low level of resistance has been observed for Maruca pod borer and pod sucking bugs, and, therefore, only limited studies have been made on these insects.

5.7.4 Genetics of New Mutants

Several mutants have been reported in cowpea and previous work has been reviewed by Fery (1985). Recently, Singh and Adu-Dapaah (1998) reported a partial sterile mutant controlled by a single recessive gene, *'pms'*. The mutant plants remained green for a longer period than the usual maturity stage, and they had thick leathery leaves with a few fleshy 1- to 3-seeded pods with gaps, and about 77% viable pollen, indicating partial male as well as partial female fertility. The homozygous recessive *(pms pms)* plants bred true for partial sterility. Adu-Dapaah et al. (1999) also reported a fasciated mutant, which was observed in an F_4 population of a cross TVu 3000×IT82D-604. The mutant plants were both male and female sterile and exhibited crumpled petals and sepals, rosette branching, and abnormal stigmas ranging in number from zero to two. Genetic study showed that this trait was controlled by a single recessive gene, which was designated as *'fa'*. Odeigah et al. (1996) reported several induced mutants, of which four were male sterile and female fertile—and two mutants were completely sterile. All six mutants showed a monogenic recessive inheritance.

5.7.5 Genetics of Leaf, Pod, and Seed Types

Inheritance of leaf, pod, and seed traits have been reviewed before (Fery, 1980, 1985; Fery and Singh, 1997). Recently, Aliboh et al. (1996) studied the inheritance of inverted V-shaped mark on leaves, pod dehiscence, and dry pod color in crosses involving wild, weedy, and cultivated varieties of cowpea. The segregation pattern in F_1, F_2 and backcross data indicated monogenic dominant inheritance for all three traits. The gene symbols *Vsm, Dhp,* and *Bk-2* were assigned for the V-shaped leaf mark, pod dehiscence, and black dry pod color, respectively. Kehinde and Ayo-Vaushan (1999) and Singh and Ishiyaku (2000) reported inheritance of seed coat texture in cowpea and indicated the involvement of two pairs of genes for this trait. The crosses between smooth and rough seed texture segregated into 3 smooth: 1 rough. However, the crosses involving white rough seed×brown rough seed showed a complementary gene action. The F_1 was smooth and F_2 segregated in 9 smooth: 7 rough ratio. This was supported by the backcross data. The gene symbols rt_1 and rt_2 were assigned for rough testa. Rough seed coat texture is an important trait in West and Central Africa because it facilitates removal of seed coat for certain food preparations.

5.7.6 Genetics of Photosensitivity

The days to flowering in photosensitive cowpea varieties is controlled by the day length during the growing season. Sene (1971) concluded that the short day response (photosensitivity) was dominant over the photo-insensitive response, but this was due to evaluation of the progenies during the long days. Ishiyaku and Singh (2001) observed that all photosensitive cultivars not only flower early but also become extremely dwarf when day lengths are less than 12.5 h. The dwarfing under short day length was observed to be a pleiotropic effect of the photosensitivity gene, as it showed monogenic recessive inheritance that is completely associated with photosensitivity. The gene symbol *'ps'* was assigned to it. This is the first report indicating the effect of photoperiod on the vegetative growth of plants. All earlier reports linked photosensitivity with reproductive stage only.

5.7.7 Genetics of Drought Tolerance

Considerable progress has recently been made in genetics of drought tolerance in cowpea. Mai-Kodomi et al. (1999a) reported simple inheritance of drought tolerance in cowpea. Using a box screening method, Singh et al. (1999a) identified two types of shoot drought tolerance. Type 1 plants stayed green for a long time after withholding the water, and the plants died as a whole with continued dry conditions. In contrast, the Type 2 plants stayed alive for a much longer period, but they did not die as a whole with continued dry conditions. They mobilized moisture from lower leaves to keep the growing tips alive for a longer time, and, thus, the plants dropped lower leaves first and dried upward slowly such that when watering was resumed, they recovered well. Both Type 1 and Type 2 drought tolerance are inherited as monogenic dominant traits. The F_1 crosses between them showed dominance of Type 1 and F_2 segregated into 3 Type 1: Type 2, suggesting that these are alleles at the same locus. The gene symbols *Rds1* (resistance to drought stress) and *Rds2* were assigned for these traits. This is the first report of monogenic inheritance of drought tolerance in plants. The simple inheritance was observed because of simplified screening methods and selective screening for shoot drought tolerance only.

As the plants sense drought stress, the gene for controlling stomata is turned on and closes them to preserve the moisture. Menendez and Hall (1996) studied the heritability of carbon isotope discrimination (DELTA), which may be a useful selection criterion for drought adaptation in cowpea. Broad-sense heritability for DELTA in two crosses (TV×309×Prima and TV× 309×CB 46) was 0.47 and 0.33, respectively, indicating intermediate level of genetic variability for this trait. Ten cDNAs of genes that were induced by dehydration stress were cloned by differential screening from drought tolerant cowpea variety (Luchi et al., 1996). The clones were collectively named CPRD (cowpea clones responsive to dehydration).

5.7.8 Genetics of Quantitative Traits and Heterosis

A large number of studies on inheritance of quantitative traits and heterosis have been reported (Fery, 1985). Damarany (1994) published information on heritability and genetic

advance for 13 characters in cowpea. Broad sense heritability for seed weight/plant was 94.4%, for pods/plant was 85.9%, and for 100 seed weight it was 83.3%. Genetics of pod yield and its components were studied in F_2 and backcross populations of a cross involving two vegetable cowpea varieties, UCR 193 and IT81D-1228–14, by Pathmanathan et al. (1997). The broad sense heritability for pod weight was 84%, and the narrow-sense heritability was 75%, indicating good genetic variability for effective selection. Menendez and Hall (1996) studied the heritability of harvest index in two crosses (TVx 309×Prima and TVx 309×CB 46). The broad sense heritability for this trait was 0.38 and 0.58 in the two crosses, respectively. Sangwan and Lodhi (1995) studied heterosis for yield and yield components in 25 crosses involving 11 cowpea varieties. Better parent heterosis ranged from 28.8% to 84.0% for seed yield/ha. The heterosis up to 81.6%, 35.65%, 20.4%, and 36.3% over better parent was observed for pod/plant, pod length, seed/pod, and seed weight/plant, respectively. Hybrid Fos-1×Co1, Fos2×EC 4216, and EC 4216×C28 were most promising. Arvindhan and Das (1996) reported 215% heterosis for seed yield in the cross CS 55×CO4. Bhor et al. (1997) studied P_1, F_1, and F_2 populations of 14 crosses and observed 63.8% better parent heterosis for seed yield in the cross V240×VCM8. They further observed that the heterosis was 4.3% for plant height and 91.52% for days to maturity. They observed that progeny derived from crosses showing high heterosis also showed high inbreeding depression, indicating the importance of nonadditive gene action. Bhushana et al. (2000) estimated heterosis for several traits in 36 hybrids. They observed a mid-parental heterosis of 171.5% for number of secondary branches/plant, 11.5% for pods/plant, 105.3% for seed yield/plant, 75.5% for primary branches/plant, 30.31% for pod length, and 20% for 100 seed weight. They also observed −15.9% heterosis for days to 50% flowering. Ponmariammal and Das (1996) and Arvindhan and Das (1996) also reported heterosis for fodder yield. The highest heterosis (121%) was recorded for the hybrid UPC9201 CO5.

High values for heterosis indicates good genetic diversity among cowpea varieties used in these studies, indicating the possibility of isolating high-yielding transgressive segregates from hybrid populations. However, the estimates for heterosis in most cases is from space planted F_1 hybrids, which may not be a true index of performance under normal plant population used for the commercial crop. Therefore, there is a need to estimate heterosis under recommended plant population for maximum yield of cowpea. This will require making a large number of pollinations to obtain sufficient quantity of F_1 seeds to test under normal density.

5.8 LINKAGE MAPPING

Studies on linkage and mapping are very few in cowpea. Early researchers observed linkage for some traits but did not follow through for further confirmation (Fery, 1985). Uguru and Ngwuta (1995) reported the first detailed linkage map in cowpea. From the genetic analysis of F_1, F_2, and F_3 populations derived from the crosses involving cowpea variety AN-14-D with purple calyx, purple petal, and purple pod and varieties An-36-F and AE-36-W, which were nonpigmented. They observed linkage between the three traits, with calyx and petal color most tightly linked (0.576±.009 cm). Another report on

linkage was published by Githiri et al. (1996), who identified genes on four linkage groups as indicated below:

	Linkage Group	Traits Involved
1.	*Sw→Fbc*	*Sw*=swollen base, *Fbc*=cream flower bud
	41±4.8	
2.	*Pus→Pub→Cbr*	*Pus*=Purple stem, *Cbr*=cocoabrown pod color, *Pub*=purple pods
	4±1.5 30±5.7	
3.	*Pod→Ndt→Hg→Bpd*	*Pod*=Purple peduncle, *Ndt*=nondeterminate, *Hg*=erect plant
	26±28 26±2.8 24±9.5	*Bpd*=Branched peduncle
4.	*Put→Bk*	*Put*=purple pod tips, *Bk*=grey black pod
	19±2.4	

They used F_2 data from four crosses to estimate the recombination frequencies, which needs further confirmation using backcross and F3 data. Kehinde et al. (1997) studied segregation patterns of 12 loci in F_2 and backcross populations and identified five linkage groups. The first linkage group comprised five genes [Pg (nodal pigmentation), Pf (purple flower), Pc (smooth seed coat), Na (narrow eye), and Br (brown seed coat)] with the probable order "Pg→Na→B→Pc→Pf." The second linkage group was Bpd (branched peduncle)→Bp (brown dry pod)→Dhp (pod dehiscence). The third linkage group consisted of Crl (crinkled leaf)→(sessile leaf). The hastate leaf (Ha) and septafoliate leaf, spt, showed independent segregation from others showing different linkage groups (four and five).

DNA marker-based (RFLP and RAPD analysis) genetic map of cowpea was first reported by Fatokun et al. (1993). This contained 92 markers with a span of 717 cM of the genome from a cross between IT84S-2246–4 and TVu 963. Recently, Menendez et al. (1997) published another genetic map consisting of 181 loci, comprising 133 RAPDs, 19 RFLPs, 25 AFLPs, 3 morphological or classical markers, and a biochemical marker (dehydrin). These markers identified 12 linkage groups spanning 97 cM, with an average distance of 6.4 cM between markers. Myers et al. (1996) identified one RFLP marker, to be tightly linked to the aphid resistance gene (Raci). Recently, Ouedraogo et al. (2001) have identified three AFLP markers tightly linked to Striga resistance gene Rsg 2–1 and six AFLP markers linked to Striga resistance gene Rsg 4–3, setting the stage for marker-assisted selection (MAS) in cowpea.

Ouedraogo et al. (2002) constructed an improved genetic linkage map for cowpea. This map consists of 11 linkage groups (LGs), spanning a total of 2670 cM, with an average distance of 6.43 cM between markers. This is based on the segregation of various molecular markers and biological resistance traits in a population of 94 recombinant inbred lines (RILs) derived from the cross between 'IT84S-2049' and '524B'. A set of 242 molecular markers, mostly amplified fragment length polymorphism (AFLP), linked to 17 biological resistance traits, resistance genes, and resistance gene analogs (RGAs) were scored for segregation within the parental and recombinant inbred lines. These data were used in conjunction with the 181 random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), AFLP, and biochemical markers

previously mapped to construct an integrated linkage map for cowpea. In addition to the construction of a new map, molecular markers associated with various biological resistance and tolerance traits, resistance genes, and RGAs were also placed on the map, including markers for resistance to S. gesnerioides races 1 and 3, CPMV, CPSMV, B1CMV, SBMV, Fusarium wilt, and root knot nematodes. These markers will be useful for the development of tools for marker-assisted selection in cowpea breeding, as well as for subsequent map-based cloning of the various resistance genes.

5.9 COWPEA BREEDING

Recent reviews by Singh et al. (1997), Hall et al. (1997), and Singh et al. (2002) have described cowpea breeding programs and their progress in different regions of the world. IITA continues to be the center of excellence for cowpea research. However, recently cowpea improvement programs at the University of California, Riverside, and EMBRAPA, Brazil, have been strengthened and expanded. Significant research on various aspects of cowpea improvement is also being done in Nigeria, Burkina Faso, Senegal, Mali, and India, and to a lesser extent in a number of other countries.

5.9.1 Production Constraints

Cowpea yields are generally low due to several biotic and abiotic constraints as well as due to cultivation of cowpea as an intercrop with cereals in marginal environments, where soils are infertile and rainfall is scanty. Under intercropping, the tall growing cereals shade cowpea, as well as compete for moisture and nutrients, and cause severe reduction in cowpea yields. Most of the cowpea is grown without use of fertilizers and plant protection measures, which causes poor growth and severe yield reduction due to pest damage.

5.9.2 Biotic Constraints

Several diseases, insect pests, and parasitic plants attack cowpea. The major diseases are anthracnose; web blight; brown blotch; Cercospora leaf spot; *Septoria;* scab and *Macrophomina* caused by fungi; bacterial pustule and bacterial blight caused by bacteria; and cowpea yellow mosaic, cowpea aphid borne mosaic, blackeye cowpea mosaic, cowpea servere mosaic, and southern bean mosaic caused by viruses. Nematodes are important in some areas, and parasitic weeds such as *Striga gesnerioides* and *Alectra vogelii* are important in Africa. *Striga* causes severe damage to cowpeas in the Sudan savanna and Sahel of West Africa, whereas *Alectra* is more prevalent in the Guinea and Sudan savannas of Central Africa. *Alectra* is also widespread in Eastern and Southern Africa, but *Striga* is not a problem there. *Striga* infection in cowpea is more devastating in areas with sandy soils, low fertility, and low rainfall. Both parasites are difficult to control because they produce large number of seeds, and up to 75% of the crop damage is done before they emerge from the ground. The major insect pests of cowpea are aphid *(Aphis craccivora),* thrips *(Megalurothrips sjostedti),* Maruca pod borer *(Maruca vitrata),* a complex of pod sucking bugs (*Clavigralla* spp., *Acanthomia* spp., *Riptortus* spp.), and

the storage weevil *(Callosobruchus maculates)*. Of these, thrips and *Maruca* cause major damage in Sub-Saharan Africa. There are some locationspecific insect pests, such as *Lygus* in the Americas, bean fly in Asia and East Africa, and ootheca beetles in wetter regions of the tropics.

5.9.3 Abiotic Constraints

Cowpea may suffer from erratic rainfall in the beginning and toward the end of the rainy season, which causes substantial reduction in grain yield as well as biomass production. Early maturing cultivars escape terminal drought, but if exposed to intermittent moisture stress during the vegetative or reproductive stages, they perform very poorly. Cowpea is inherently more drought tolerant than other crops, but it still suffers considerable damage due to frequent drought in the Sahelian region, where rainfall is scanty and irregular.

5.9.4 Regional Preference for Diverse Cowpea Varieties

Even though cowpea is one crop, the diverse regional preferences make the breeding objectives very challenging. For example, only white- and brown-seeded varieties with rough seed coats are preferred in West and Central Africa because of the ease of removing the seed coats for local food preparations. On the other hand, red- or brown-seeded varieties with smooth seed coats are preferred in East and Southern Africa and parts of Central and South America, where cowpea is used as boiled beans for which removal of the seed coat is not desirable. Cuba and some of the other countries in Central America like black-seeded cowpea varieties as a substitute for black beans as local delicacies. Similarly, there is a need for late-maturing dual-purpose cowpea varieties in East and Southern Africa, where cowpea leaves are used as an important vegetable—and in West Africa, where cowpea stovers are important fodder for the livestock—but most countries would like to have early- and medium-maturing varieties because cowpea is grown in low rainfall areas. Most of the Asian countries grow cowpea for green pods as a vegetable, and some grow them exclusively for fodder. Thus, there is a need for developing a diverse set of cowpea varieties, including for sole and intercropping.

5.9.5 Breeding Strategy

Among all the legumes, cowpea has the maximum diversity for plant type, growth habit, maturity, and seed type, and therefore, it offers an unique opportunity to cowpea breeders to design and develop specific plant types with desired maturity to suit different cropping systems and agroecological niches (Singh and Sharma, 1996). Therefore, the general strategy is to develop a range of cowpea varieties differing in growth habit and maturity, seed type, and for sole crop and intercrop in different agro-ecologies. IITA's cowpea breeding, in collaboration with national programs, has focused on developing the following types of varieties:

1. Extra-early maturing (60 to 70 days) nonphotosensitive grain type, for use as sole crop in multiple cropping systems and short rainy seasons
2. Medium-maturing (75 to 90 days) nonphotosensitive grain type, for use as sole crop and intercrop

3. Late-maturing (85 to 120 days) nonphotosensitive dual-purpose (grain+leaf) types, for use as sole crop and intercrop
4. Photosensitive early-maturing (70 to 80 days) grain types, for intercropping
5. Photosensitive and photo-insensitive medium-maturing (75 to 90 days) dual purpose (grain+ fodder) types, for intercropping
6. Photosensitive late-maturing (85 to 120 days) fodder type, for intercropping
7. High-yielding, bush-type vegetable varieties
8. Desirable seed types and seed colors, with high protein content and low cooking time
9. Resistance to major diseases, insect pests, and parasitic weeds
10. Tolerance to drought, low pH, and adaptation to sandy soils and low fertility

5.9.6 Breeding for Improved Early and Medium Maturing Grain-Type Varieties

Initially, the major focus of IITA's cowpea improvement program was to develop extra-early erect-type varieties with high yield potential, and considerable progress was made (Singh and Ntare, 1985). These varieties have erect plant type and determinate growth habit (Figure 5.1), and these are collectively called 60-day cowpeas, which yield about 2t/ha within 60 to 65 days. They fit well in the short rainy seasons and specific niches in cereal systems. With good management, and heavy pod load, these varieties shed most of the leaves, and, therefore, the grain yields of these varieties

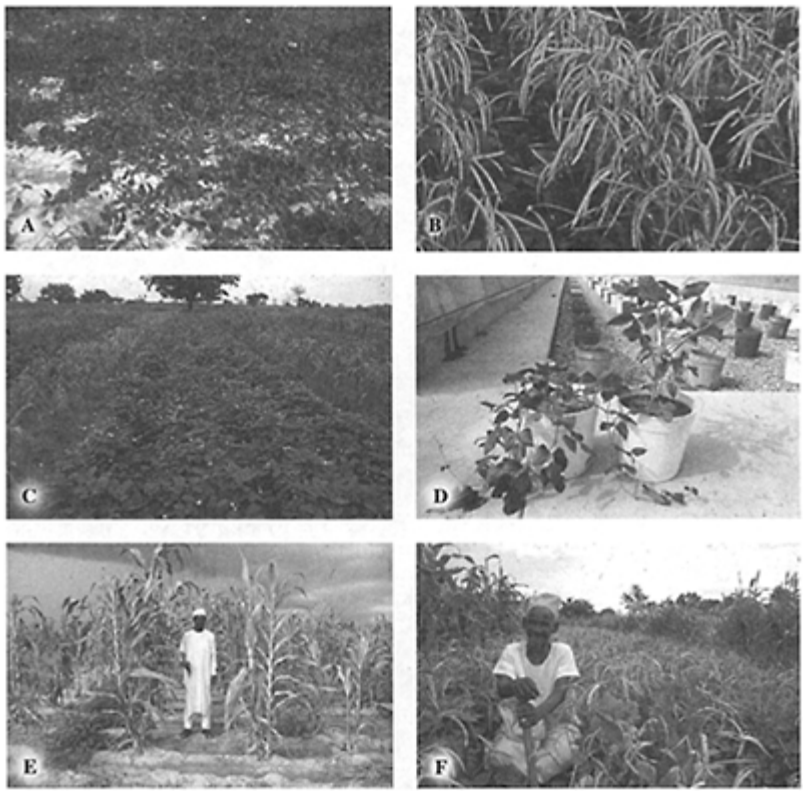

Figure 5.1 Local and improved cowpea varieties and cropping systems: A=local variety (spreading); B= improved early grain type (erect early); C=improved medium dual-purpose (semi-erect); D= improved vegetable cowpea; E=traditional intercrop; F=improved intercrop.

are high, but fodder yields are low. This is also because these varieties have short growth periods for biomass accumulation and greater harvest index.

Most of the national programs and IITA have also developed medium-maturing varieties, which have semi-erect growth habit with 75 to 85 days maturity, and they fit well in rotation with other crops. Over the years, a great deal of progress has been made, and about 68 countries have released improved IITA cowpea varieties for general cultivation (Singh et al., 1997) in addition to varieties developed by the national programs. Some of the important and popular 60-day varieties are IT82E-16, IT82E-18, IT82D-889, IT93K-452–1, and IT97K-499–35, and medium-maturing varieties are

IT86D-721, IT88D-867–11, and IT90K-277–2. The performance of some of the recently developed grain-type varieties is indicated in Table 5.8, of which IT98K-491–4, IT99K-1399, and IT97K-499–38 yield more than 2t/ha, and these have combined resistance to many diseases, insect pests, and *Striga*. The low yields without spray of insecticides are due to severe damage by Maruca pod borer, for which good sources of resistance are not available.

Table 5.8 Performance (Yield Kg/ha) of Promising Early Cowpea Varieties at Minjibir (Kano) in the Sudan Savanna, 2002

	2 Sprays		No Spray*	
Variety	**Grain**	**Fodder**	**Grain**	**Fodder**
IT98K491–4	2438	1958	377	2708
IT99K-1399	2472	1625	52	4083
IT97K-499–38	2031	1083	300	1917
IT99K-494–3	1918	542	606	2625
IT98K-589–2	1880	1833	108	3833
IT98K-503–1	1876	1458	244	2458
IT98K-463–8	1654	875	241	3042
IT97K-568–18	1556	1458	496	3542
IT99K-494–6	1296	500	634	1958
Danila	1548	1458	53	3753
Mean of 30	1428	900	252	2566
SED	439	486	190	690

* The low yield without spray of insecticide is due to severe damage caused by Maruca pod borer.

5.9.7 Imroved Dual-Purpose Cowpea Varieties

Cowpea continues to be a major source of food and fodder in the dry savannas of West and Central Africa. Almost all of the farmers have livestock and intercrop two types of cowpea varieties in alternate rows with millet or sorghum in the same field—one for grain and the other for fodder. Both are spreading types, but the grain type is early maturing (80 to 85 days) and the fodder type is late maturing (100 to 120 days). The grain cowpea and millet are harvested at the end of August and beginning of September, while the late cowpea is left in the field until the onset of dry season (October to November). The farmers wait until the cowpea leaves show signs of wilting before they cut the cowpea plants at the base and roll the plants into bundles with all leaves still intact. Those bundles are kept on rooftops or in tree forks for drying and are sold in the peak dry season when prices are high. In case there are rains in October and November, the

fodder-type cowpeas produce some grain as well (Mortimore et al., 1997; Singh and Tarawali, 1997). Even though this is a good system, and it utilizes rainfall from May to November, the overall productivity is low because the grain-type varieties flower and pod under the shade of millet, and the fodder-type varieties are often affected by drought due to early cessation of rains. Therefore, an ideal cowpea variety for this system would be a dual-purpose type with semi-determinate growth habit and intermediate maturity (85 to 95 days) so that it flowers in September when millet has been harvested and becomes ready for first picking at the onset of the dry season. Several such varieties have been developed that yield more than 2t/ha grain and 2 to 5t/ha fodder (Table 5.9).

5.9.8 Bush-Type Vegetable Cowpea Varieties

Several countries grow cowpea as a vegetable crop. The most preferred types are the yardlong cowpeas with fleshy tender pods, but these cultivars need staking to keep pods from touching the ground and rotting, which involves extra cost and, thus, restricts the area under cultivation. Bush-type vegetable cultivars with 30-cm long succulent pods have been developed, such as IT81D-1225–10, IT81D-1228–14, IT81D-1225–15, and IT86D-880, which yield up to 18 t ha-1 green pods with 3 to 4 pickings starting at 45 days after planting. These cultivars have semi-erect growth habit with extra-long peduncles (40 to 50 cm long) protruding well over the canopy and holding the pods above the ground. Picking green pods periodically reduces the weight on peduncles, and they remain upright all the time. Frequent picking also stimulates further flowering and podding on the

Table 5.9 Performance (Yield Kg/ha) of Promising Dual-Purpose Cowpea Varieties in the Sudan Savanna in 2002

	2 Sprays High Population		2 Sprays Low Population	
Variety	**Grain**	**Fodder**	**Grain**	**Fodder**
IT98K-537–4	2346	2903	1559	1069
IT99K-213–13–1	2146	4481	1455	2416
IT99K-687	2008	3702	1270	1527
IT99K-23–1	1838	3424	1179	1944
IT99K-262	1788	5038	1018	3388
IT99K-7–21–2-2	1748	3674	1565	2291
IT99K-2 16–2 1–2	1679	3340	1016	2763
IT99K-21 6–24–2	1476	4064	1118	3124
IT99K-7–16–1	1578	4620	1268	2513
IAR-1696	913	5177	1330	3096

Kananado	1094	4147	878	3276
Borno Local	636	4481	598	1938
Mean of 25	1429	3838	1046	2320
SED	322	649	297	486

same peduncles, which ensures a continuous supply of green pods for a 6- to 7-week period after the start of picking, provided soil moisture is not limiting (Singh et al., 2003a).

5.9.9 Breeding for Resistance to Diseases

Several fungal, viral, and bacterial diseases, nematodes, and parasitic plants *(Striga and Alectra)* attack cowpea. Cowpea bacterial blight and aphid borne mosaic are the most serious and widespread diseases in the Sudan savanna and Sahel. Good sources of resistance to all these diseases and *Striga* and *Alectra* have been identified, and several varieties with combined resistance to these have been developed (Singh et al., 1997a; van Boxtel et al., 2000; Mustapha et al., 2000; Singh and Singh, 1990; Singh and Emechebe, 1997; Singh, 2002).

Using a combination of field and laboratory screening, a number of new sources of resistance to major diseases were developed by several workers. Latunde-Dada et al. (1999) studied the mechanism of resistance to anthracnose in Tvx 3236 cowpea. In this variety, the initially injected epidermal cells underwent a hypersensitive response, restricting the growth of the pathogen. The phytoalexins ‘kievitone’ and ‘phaseollidin’ accumulated more rapidly in the stem tissue of Tvx 3236 compared to the sucessible variety. Lin et al. (1995) screened 131 cowpea varieties by artificially inoculating them with *Cercospora cruenta (Mycosphaerella cruenta)*, from which 15 varieties were identified immune and 7 were resistant. Singh (1998) identified and developed several cowpea lines with resistance to Cercospora, smut, rust, Septoria, scab, Ascochyta blight, and bacterial blight (Table 5.10). Some of the varieties that showed multiple resistances were IT97K-1021–15, IT97K-556–4, and IT98K-476–8. Wydra and Singh (1998) screened 90 cowpea breeding lines and identified IT90K-284–2, IT91K-93–10, and IT91K-118–20 to be completely resistant to 3 virulent strains of bacterial blight. Eight varieties were resistant to 2 strains and 2 varieties were resistant to 1 strain. All the remaining varieties were susceptible to bacterial blight. Dos Santos et al. (1997) screened 156 cowpea varieties under field infestation with smut and identified 3 highly resistant ones. Nakawuka and Adipala (1997) identified Kvu 46, Kvu 39, and Kvu 454 to be resistant to scab in Uganda. Rodriguez et al. (1997) found L-198 and CNx 377–1E to be resistant to *Macrophomina*. Uday et al. (1996) identified V-265 also to be resistant to *Macrophomina*. In an interesting study, Zohri (1993) artificially inoculated 16 cowpea varieties with *Aspergillus flavus* to monitor aflatoxin production. He found that two cowpea varieties from IITA, IT82E-16 and IT81D-1032, did not support *Aspergillus* growth, and therefore, no aflatoxin production was

Table 5.10 Source of Resistance to Major Diseases in Cowpea

Diseases	Sources of Resistance	Reported by
Anthracnose	TVx 3236	Latunde-Dada et al. 1999
Cercospora	IT89KD-288, IT97K-1021 -15	Singh, 1998
	IT97K-463–7, IT97K-478–10	
	IT97K-1069–8, IT97K-556–4	
Smut	IT97K-556–4, IT95K-1090–12	Singh, 1999
	IT95K-1091–3, IT95K-1106–6	
	IAR-48, IT97K-506–6	
Rust (Uromyces)	IT97K-1042–8, IT97K-569–9	Singh, 1999
	IT97K-556–4, IT97K-1069–8	
	IT95K-238–3, IT97K-819–118	
	IT90K-277–2, IT97K-1021–15	
	IT96D-610, IT86D-719	
Septoria	TVu 12349, Tvu11761, IT95K-398–14	Singh, 1997, 1998, 1999
	IT90K284–2, IT95K-1090–12	
	IT97K-1021–15, IT98K-205–8	
	IT98K-476–8, IT97K-819–118, IT95K-193–12	
Scab.	TVu 1234, IT95K-1090–12, IT98K-476–8, IT97K-1069–8	Singh, 1997, 1998, 1999
	TVx 3236, IT95K-398–14	
	IT97K-1021–15, IT95K-1133–6	
Ascochyta	TVu 11761	Singh, 1997
Bacterial Blight	IT95K-398–14, IT95K-193–12	Singh, 1998, 1999
	IT81D-1228–14, IT95K-1133–6	
	IT97K-556–4, IT97K-1069–8, IT90K-284–2, IT91K-93–1,	
	IT91K-118–20	

observed on those varieties. This indicates the possibility of breeding for resistance to *A. flavus* in cowpea. Using biotechnological tools, these genes can also be identified, cloned,

and transferred to other crops that have a serious problem of aflataxin, like groundnut and maize.

5.9.10 Resistance to Nematodes

Several sources of resistance to nematodes were identified, including some of the improved breeding lines with high yield potential (Rodriguez et al., 1996; Robert et al., 1996; Fery and Dukes, 1995a; Ehlers et al., 2000a; Singh, 1998). Some of the varieties with high yield and nematode resistance are IT849–2049, IT89KD-288, IT86D-634, IT87D-1463, IT95K-398–14, IT96D-772, IT96D-748, IT95K-222–5, IT96D-610, IT87K-818–18, and IT97K-556–4. Among these varieties, IT89KD-288 was found to be resistant to four strains of *M. incognita* in the U.S. (Ehlers et al., 2000a). Singh et al. (1996; Singh, 1998) found IT89KD-288 to be high yielding and highly resistant to nematodes in the trials conducted at Kano (Nigeria), where nematode attacks are very severe in the dry season planting when irrigation is used. IT89KD-288 was taken by one farmer in 1994, and through farmer-to-farmer diffusion, more than 10,000 farmers grew this variety in 2000 because of its nematode resistance and high yield in the dry season. Cowpea cultivation in the dry season was not possible before, because all the local cowpea varieties were susceptible to nematodes.

5.9.11 Resistance to Virus

Singh and d'Hughes (1999) reported several cowpea breeding lines to be completely resistant to cowpea yellow mosaic, blackeye cowpea mosaic, and cowpea aphid borne mosaic. Of these, IT96D-659, IT96D-660, IT97K-1068–7, and IT95K-52–34 were most promising in terms of resistance and yield potential. Bashir et al. (1995) screened several cowpea varieties from IITA and observed that IT86F 2089–5, IT86D-880, IT90K-284–2, IT90K-76, IT86D-1010, and IT87D-611–3 were immune to blackeye cowpea mosaic. van-Boxtel et al. (2000) artificially screened 14 cowpea varieties with 3 isolates of blackeye cowpea mosaic and 10 isolates of cowpea aphid-borne mosaic virus in order to identify lines with multiple strain resistance. They observed that cowpea breeding lines IT86D-880 and IT86D-1010 were resistant to all three isolates of blackeye cowpea mosaic and five strains of cowpea aphid-borne mosaic. IT82D-889, IT90K-277–2, and TVu 201 showed resistance to one or the other of the five remaining isolates, and, thus, by using the above mentioned five cowpea varieties as parental lines, it is possible to breed new cowpea varieties with combined resistance to all 13 strains of viruses. This work is in progress at IITA.

One of the most important factors that constrain the cowpea production in the northeastern region of Brazil are the virus diseases, caused mainly by cowpea severe mosaic virus (CSMV), cowpea aphid-borne mosaic virus (CABMV) of the group potyvirus, cucumber mosaic virus (CMV) of the group cucumovirus, and cowpea golden mosaic virus (CGMV) (Lima and Dos Santos, 1988). Substantial efforts have been made in breeding for resistance to viruses, and progress has been made. Lima and Nelson (1977) identified the cultivar Macaibo as having immunity to CSMV, while Rios and das Neves (1982) confirmed the immunity of Macaibo and a new source of resistance to CSMV in line FP 7733–2, from which the variety CNC 0434 was developed (Rios et al.,

1982). This variety was recommended for cultivation in the state of Maranhão (EMBRAPA, 1986). Lima et al. (1986), in a study that involved 248 genotypes, identified four new genotypes (TVu 379, TVu 382, TVu 966, and TVu 3961) as being immune to CSMV and CABMV. Cultivars 'Cowpea 535', Dixiecream, Bunch Purple Hull, Lot. 7909-Purple, V-17, and TVu 612 were immune only to CABMV. Lima et al. (1998), in another study that involved 44 genotypes, confirmed the immunity of genotypes TVu 379, TVu 382, TVu 966, and TVu 3961 to three strains of CSMV. Dos Santos and Freire Filho (1986) screened 450 genotypes for resistance to CGMV. Of those genotypes, 57 were classified as highly resistant, including CNC 0434, TVu 612, CE-315 (TVu 2331), and BR 1-Poty. Three lines from the EMBRAPA cowpea breeding program, TE87–98–8G, TE87–98–13G, and TE87–108–6G, and two lines introduced from IITA, IT84S-2135 and IT84S-1627, were found to be resistant to CABMV and immune to CMV by the Laboratory of Virology of the Center of Agrarian Sciences of the Federal University of Ceará. Two other lines from IITA, IT85F-2687 and IT86D-716, were immune to both viruses (Rocha et al., 1996).

These resistance sources have been used in cowpea improvement in Brazil. Several varieties that have been released commercially and breeding lines that are still under evaluation were developed from crosses with the varieties CNC 0434, Macaíbo, and Tvu 612. Resistance to CSMV, CABMV, and CGMV is already incorporated in some of the released varieties like BR 10-Piaui (Dos Santos et al., 1987), BR 12-Canindé (Cardoso et al., 1988), BR 14-Mulato (Cardoso et al.,1990), BR 17-Gurguéia (Freire Filho et al., 1994), EPACE 10 (Barreto et al., 1988), Setentão (Paiva et al., 1988), IPA 206 (IPA, 1989), and BR 16—Chapeo-de-couro (Fernandes et al., 1990). Presently, crosses are being made to improve resistance to CMV.

5.9.12 Resistance to *Striga* and *Alectra*

Two parasitic flowering plants, *Striga gesnerioides* (Wild.) Vatke and *Alectra vogelii* (Benth.) cause substantial yield reduction in cowpea in the dry savannas of Sub-Saharan Africa. *Alectra* is more prevalent in the northern Guinea savanna and Southern Sudan savanna of West Africa, as well as in East and Southern Africa, whereas *Striga* is mostly found in West and Central Africa. However, both are fast spreading beyond these limits. Good progress has been made in breeding improved cowpea varieties with combined resistance to *Striga* and Alectra (Atokple et al., 1995; Berner et al., 1995; Singh and Emechebe, 1997; Singh et al., 1997a; Singh, 2002). Collaborative studies with national and regional programs have revealed the presence of five strains of *S. gesnerioides*, of which 'strain 1' is presently found in Burkina Faso, 'strain 2' in Mali, 'strain 3' in Nigeria and Niger, 'strain 4' in Benin Republic, and 'strain 5' in Cameroon. A local landrace, B 301 from Botswana, confers complete resistance to *Striga* and *Alectra* in Burkina Faso, Mali, Cameroon, Niger, and Nigeria. However, it has only moderate level of resistance to the 'strain 4' from Benin Republic. Other lines, such as IT81D-994, IT89KD-288, 58–57, and Gorom local, confer complete resistance to strains from Benin Republic and Burkina Faso. Therefore, crosses were made among the selected complementary parents, and a number of new varieties have been developed with combined resistance to *Alectra* as well as all the five strains of *Striga*. The most promising new cowpea varieties are IT93K-693–2, IT95K-1090–12, IT97K-499–35,

IT97K-499–38, IT97K-499–39, IT97K-497–2, IT97K-819–118, and IT97K-819–154, with combined resistance to *Striga* and *Alectra*. Some of these lines are also resistant to bacterial blight, aphid, bruchid, thrips, and viruses with much higher yield potential than the local varieties (Carsky et al., 2003). Most of these lines also serve as a false host for *S. hermonthica*, reducing its seed bank in the soil when grown as intercrop or in rotation with cereals.

5.9.13 Resistance to Insects

Insect-pests are a major constraint in cowpea production. Considerable progress was made in the last four years in developing cowpea varieties resistant to several insects. Pandey et al. (1995) reported TVu 908 to be resistant to leaf beetles. Singh et al. (1996) reported several improved cowpea varieties with combined resistance to aphid, thrips, and bruchid. Of these, IT90K-76, IT90K-59, and IT90K-277–2 are already popular varieties in several countries. Among the new varieties, IT97K-207–15, IT95K-398–14, and 98K-506–1 have high level of bruchid resistance (Singh, 1999a). Nakansah and Hodgeson (1995) confirmed resistance of Tvu 801 and Tvu 3000 to Nigerian aphid strain but found that the two lines were susceptible to aphids from the Philippines. Similar differential reaction to aphid has been observed in the U.S. (A.E. Hall, personal communication, 1999), indicating existence of different aphid strains. Shade et al. (1999) also reported a virulent strain of bruchid *(Callosobruchus maculatus)* that was able to cause severe damage to Tvu 2027, which is otherwise resistant to normal bruchid strain. Yunes et al. (1998) observed that the 7s-storage protein, 'vicillin', is responsible for bruchid resistance in cowpea lines related to Tvu 2027. Only low level of resistance has been observed for Maruca pod borer and pod bugs, which cause severe damage and yield reduction in cowpea. Jagginavan et al. (1995) observed cowpea lines P120 and C11 to be least damaged by Maruca, and Veeranna and Hussain (1997) found TVx 7 to be most resistant to Maraca and with high density of trichomes (21.41/mm^2). Veerappa (1998) screened 45 cowpea lines for resistance to Maruca pod borer and observed that the tolerant lines had higher phenol and tannin contents compared to susceptible lines. This is in line with the general observation that cowpea varieties with pigmented calyx, petioles, pods, and pod tips suffer less damage due to Maruca.

5.9.14 Pyramiding Available Genes for Resistance

Over the years, IITA scientists have systematically added genes for resistance in improved breeding lines, as well as some selected varieties as recurrent parents. Starting with the susceptible but high-yielding variety Ife Brown, as a base in 1973, successive improved but related varieties like TVx 3236, IT82D-716, IT84S-2246–4, IT90K-59, IT90K-76, IT97K-499–35, and IT00K-1251 have had successive additions of new genes for resistance, such that the newest varities are resistant to all the major pests except for Maruca and pod bugs (Table 5.11). These are used as varieties as well as parents in breeding programs.

5.9.15 Efforts to Develop Maruca-Resistant Cowpeas

As indicated earlier, a distant wild relative of cowpea, *V. vexillata*, has shown high level of resistance to Maruca pod borer and bruchid, but all the efforts made at IITA to transfer Marucaresistant genes from *V. vexillata* to cowpea have not been successful (Fatokun, 2002). However, Gomathinayagam et al. (1998) reported a successful susceptible cross between *V. vexillata* and cowpea and made a backcross in F_2 generation. The resulting seeds looked like the wild parent

Table 5.11 Progress in Pyramiding Genes for Resistance to Diseases, Insect Pests, and Parasitic Weeds in Cowpea*

Pest/Disease Factor	Variety							
	Ife Brown	TVx3236	IT82D-716	IT84S-2246	IT90K-59	IT90K-76	IT97K-499-35	ITOOK-1251
	1973	1978	1982	1984	1990	1990	1997	2000
Anthracnose	S	R	R	R	R	R	R	R
Cercospora	S	R	R	MR	R	R	R	R
Brown blotch	S	R	R	MR	R	R	R	R
Bacterial pustule	S	R	R	R	R	R	R	R
Bacterial blight	MR	MR	MR	MR	MR	MR	R	R
Septoria	S	S	S	S	S	S	R	R
Scab	S	MR	MR	MR	MR	R	R	R
Web blight	S	MR	MR	MR	MR	R	R	R
Yellow mosaic	S	S	R	R	R	R	R	R
Aphid-borne mosaic	S	R	R	R	R	R	R	R
Golden mosaic	R	R	R	R	R	R	R	R
Aphid	S	S	S	R	R	R	R	R
Thrips	S	MR	MR	MRMR	R	R	R	
Bruchid	S	S	R	R	R	R	R	R
Striga	S	S	S	S	R	R	R	R
Alectra	S	S	S	S	R	R	R	R

Nematode	S	S	S	R	R	R	R	R

* The earlier variety is one parent of the next variety. See dates in parentheses after each variety.
Note: R=resistant; MR=moderately resistant; S=susceptible.

(personal communication, 2000), further raising the question whether the original cross and the backcross seeds were true hybrids. He did not follow this work. Over the last 10 years, concerted efforts have been made by IITA in collaboration with advanced laboratories in the U.S. and Italy to transform cowpea with Bt gene for Maruca resistance. Success has not yet been achieved, but the efforts continue.

While the wide crosses and transformation of cowpea with Bt gene have not been successful, considerable progress has been made in pyramiding minor genes for field resistance to Maruca pod borer and pod bugs through conventional breeding. Singh (1999a) screened new, improved cowpea breeding lines for field resistance to major insect pests without insecticide sprays, and he observed several cowpea lines with grain yields of 500kg/ha to 856kg/ha, without any chemical protection. The local variety yielded 0 to 48kg/ha in the same trials. The most promising varieties are IT90K-277–2, IT93K-452–1, IT94K-437–1, IT97K-569–9, IT95K-222–3, IT97K-837, and IT97K-499–38. These lines are resistant to major foliar diseases, aphid, thrips, and bruchid, have pods at wide angles, and suffer less damage due to Maruca. IT94K-437–1 and IT97K-499–38 also have combined resistance to *Striga* and *Alectra*. Developed through conventional breeding approaches, the new field-resistant lines require only one or two sprays of insecticide for normal yield of 1.5 to 2.5 tons compared with five to six sprays needed for the susceptible varieties.

5.9.16 Breeding for Drought, Heat, and Cold Tolerance

Since cowpea is grown in varied environments, it encounters different types of stresses, including drought, heat, and cold temperatures. Even though cowpea is inherently more drought tolerant, the low and unreliable rainfall, particularly in the beginning and toward the end of the crop season, affects cowpea yields. Early maturing varieties escape terminal drought (Singh, 1987), but if exposed to intermittent moisture stress during the vegetative or reproductive stages, they perform equally poorly. In addition, cowpea suffers due to high temperatures in the Sahelian region. Normally, cowpea performs well in a temperature range of 23 to 28°C. If the temperatures are higher than the optimum range, cowpea plants show floral abortion and substantial yield reduction. Therefore, efforts are being made to develop improved cowpea varieties with combined tolerance to drought and heat. Good progress has been made at IITA on breeding for enhanced drought and heat tolerance. Using a simple screening method for heat and drought tolerance and root architecture, major varietal differences for all three traits have been identified and incorporated into improved lines (Singh et al., 1999a; Mai-Kodomi et al., 1999a, 1999b; Singh et al., 1999b; Singh, 2001a; Singh and Matsui, 2002). Performance of selected drought-tolerant and susceptible lines under normal moisture and under drought stress is indicated in Table 5.12, and performance of some of the improved drought-tolerant lines at Zinder, Niger Republic, with less than 400 mm rainfall is indicated in Table 5.13. Good progress has also been made at the University of California, Riverside, on water use efficiency, heat tolerance, and chilling tolerance

(Singh et al., 1999a, 1999b; Mai-Kodomi et al., 1999a, 1999b; Hall et al., 1997; Ismail and Hall, 1998; Singh, 1999a). Simple, cheap, and nondestructive screening methods for drought tolerance have been developed and used to identify and breed for drought-tolerant cowpea varieties.

Heat-tolerant lines have been developed, and heat tolerance is now better understood in cowpea than in any other crop (Singh, 1999c; Ismail and Hall, 1998). Recently the effectiveness of heat tolerance has been quantified using pairs of genetically related and unrelated lines with and without heat tolerance genes (Ismail and Hall, 1998). Singh (1999) grew 102 cowpea breeding lines at IITA Kano Station from March to May, when temperatures range from 24 to 27°C in the night and from 38 to 42°C during the day. Most of the lines showed severe flower abortion with little or no pods, and these were rated as heat susceptible. The most susceptible lines, IT97K-461–2 and IT97K-461–4, showed complete sterility with no development of pollen beyond the microspore stage. These lines are otherwise normal and very high yielding in the regular crop season (July through October), when day temperatures are below 35°C and night temperatures below 24°C. In contrast to the heat-

Table 5.12 Performance of Drought-Tolerant Lines with and without Moisture Stress

	Yield Kg/ha			
	No Moisture Stress		With Moisture Stress	
Variety	Grain	Fodder	Grain	Fodder
Drought-Tolerant Varieties				
IT98D-1399	1284	4036	1350	1086
IT98D-131–1	1665	3154	1473	1030
IT97K-568–19	1448	5817	1533	1948
IT98K-452–1	1442	1976	1294	1002
IT00K-1147	1751	1447	1529	1141
IT00K-1149	1554	1818	1483	1130
Drought-Susceptible Varieties				
IT93K-734–2	1484	919	1115	778
IT95K-238–3	1269	1197	766	835
TVu 7778	938	1392	564	863
SED	261	432	261	432

Table 5.13 Performance of Improved Drought-Tolerant Cowpea Varieties at Zinder, Niger Republic with About 400 mm Rainfall

Variety	Yield Kg/ha	
	Grain	Fodder
IT97K-1021–2–4	1574	1893
IT97K-837	1491	1559
IT98K-205–15	1385	1642
IT97K-819–118	1362	1865
IT95K-207–15	1351	1642
IT98K-506–1	1233	1141
Danila	866	2366
Mean of 40	1011	1543
SED	235	354

susceptible lines, the heat-tolerant lines had normal pollen, good pod set, and normal grain yield. The best heat-tolerant lines were IT97K-472–12, IT97K-472–25, IT97K-819–43, and IT97K-499–38.

A dehydrin gene involved in chilling tolerance during the seedling stage has been identified (Ismail et al., 1997, 1999) and mapped using recombinant inbred lines (Menendez et al., 1997). The role of the dehydrin in chilling tolerance has been confirmed using near-isogenic lines (Ismail et al., 1999), and efforts are under way to understand the mechanism involved in the control of its expression.

5.9.17 Breeding for Enhanced N-Fixation and Efficient Use of Phosphorus

Significant variation in cowpea rhizobium strains has been observed for nodulation in cowpea (Mandal et al., 1999), but the local rhizobia invariably outpopulates the introduced strains. Therefore, in recent years, major efforts are concentrated to exploit genetic variability in cowpea as a host for effective nodulation and nitrogen fixation (Buttery et al., 1992). Graham and Scott (1983) observed major genetic differences for nodulation and dry matter and N accumulation among 12 cowpea varieties. They also observed a significant relationship between total N and seed yield and nodule weight. Mandal et al. (1999) also observed significant varietal differences in cowpea for nodule number and nodule weight, as well as for nitrogenase activity, indicating a good possibility of breeding improved cowpea varieties with enhanced N-fixation. Sanginga et al. (2000) screened 94 cowpea lines and observed major varietal differences in cowpea for growth, nodulation, and arbuscular mycorrhizal fungi root infection as well as for performance under low and high phosphorus. The improved cowpea variety IT86D-715

showed equally good growth under low, as well as high, phosphorus levels. It also showed better N-fixation than others. Based on its adaptability to grow in low P soils and overall positive N balance, they recommended cultivation of IT86D-715 cowpea variety in soils with low fertility. Kolawale et al. (2000) screened 15 cowpea varieties for tolerance to aluminum and to determine the effect of phosphorus addition on the performance of Al-tolerant lines. The results indicated IT91K-93–10, IT93K-2046–1, and IT90K-277–2 cowpea varieties to be tolerant to aluminum, and they gave higher response to phosphorus fertilization when grown in soils with aluminum toxicity problems. Singh and Ajeighe (1998) evaluated improved cowpea varieties under low and high fertility, and they also observed major varietal differences. They found IT96D-772, IT96D-739, IT96D-740, and IT96D-666 cowpea varieties to be good performers under low, as well as high, fertility, whereas most other varieties were poor in poor fertility and good in good fertility. These studies further indicate a good possibility of developing improved cowpea varieties with enhanced nitrogen fixation and higher yields under low phosphorus, as well as in soils with aluminum toxicity. There is a need for closer interactions among cowpea breeders, soil scientists, and soil microbiologists.

5.9.18 Breeding for Improved Nutritional Quality

Cowpea is a major source of protein, minerals, and vitamins in the daily diets of the rural and urban masses in the tropics, particularly in West and Central Africa where it complements the starchy food prepared from cassava, yam, sorghum, millet, and maize. Systematic efforts have just begun at IITA and a few other institutions to develop improved cowpea varieties with enhanced levels of protein and minerals combined with faster cooking and acceptable taste. Singh (1999b) screened 52 improved and local cowpea varieties to estimate the extent of genetic variability for protein, fat, and minerals. On a fresh weight basis (about 10% moisture), the protein content ranged from 20 to 26%, fat content from 0.36 to 3.34%, iron content from 56 to 95.8 ppm, and manganese content from 5 to 18 ppm. The improved cowpea varieties IT89KD-245, IT89KD-288, and IT97K-499–35 had the highest protein content (26%), whereas the local varieties like Kanannado, Bauchi early, and Bausse local had the lowest protein content (21 to 22%). One local variety, IAR 1696, had high protein content (24.78%) and high fat content (3.28%), as well as high iron content (81.55 ppm). Similarly, an improved variety, IT95K-686–2, had high protein content (25%), high fat content (3.3%), and high iron content (76.50 ppm). Appropriate crosses have been made to study the inheritance of protein, fat, and iron contents and to initiate a breeding program for improving these quality traits. In another experiment, various physical properties of selected cowpea varieties were determined. The relative density of cowpea seed ranged from 1.01 to 1.09, hardness (crushing weight) ranged from 3.96 kg for IT89KD-288 to 8.4 kg for Aloka local.

Significant genetic differences have been observed for all the physical properties, such as seed density, swelling properties, seed hardness and cooking time, and protein content (Singh, 2001b). The amount of water absorbed was highest in Kanannado and IT98K-813–21 and lowest in IT90K-277–2 and IT95K-207–15. Aloka local appeared to have the hardest seed, with 9 kg crushing strength compared to 3.7 kg for TVu 12349. The seed coat content ranged from 5.7% in IT95K-207–15 to 13.8% in TVu 12349 and cooking

time ranged from 27.5 minutes for IT90K-277–2 to 57.5 minutes for Aloka local. The smooth-seeded variety had more seed coat content than the rough-seeded varieties. The estimates for broad sense heritability indicated that most of the traits were highly heritable. The nutritional properties of selected varieties are indicated in Table 5.14.

The calcium content ranged from 0.1% in IT90K-277–2 to 0.18% in Aloka. Similarly, the range for iron was from 103 ppm in IT90K-277–2 to 149 ppm in IT95K-1113–3, and the range for zinc was from 34 ppm in IT97K-1105–5 to 48 ppm in Aloka. It was interesting to note that Aloka had

Table 5.14 Nutritional Properties of Selected Cowpea Varieties

Variety	Protein %	Calcium %	Iron ppm	Zinc ppm
IT90K-277–2	23.4	0.10	103	39
Dan IIa	27.3	0.14	137	39
Aloka	23.6	0.18	143	48
IT93K-452–1	22.8	0.14	129	39
IT98K-813–21	24.4	0.14	134	39
IT95K-1113–3	25.7	0.12	149	36
TVU 12349	27.4	0.12	118	37
Kanannado	25.5	0.11	118	37
IT95K-207–15	14.7	0.13	129	39
IT97K-1105–5	28.1	0.14	145	34
SED	0.4	0.01	1.2	2.9

the hardest seed, and it took the maximum time to cook. It also had the maximum amount of calcium, iron, and zinc. The seed hardness was positively correlated with calcium content (r= 0.70), iron content (r=0.29), and zinc content (r=0.40). High amounts of calcium, iron, and zinc are desirable from a nutritional standpoint. However, they may increase the seed hardness and cooking time. Soaking of the seeds before cooking reduces cooking time.

Seed hardness was positively correlated with cooking time. There have been earlier reports on the extent of genetic variability for quality traits in cowpea. Hannah et al. (1976) reported high methionine content in Tvu 2093 and Bush Sitao (3.24–3.4 mg/g) dry seeds compared to 2.75–2.88 mg/g seeds of the check variety, G-81–1. del Rosario et al. (1980) observed the highest trypsin inhibitor activity in winged bean and lima bean and the lowest activity in mungbean and rice bean, whereas the trypsin inhibitor values for cowpea were intermediate. Fashakin and Fasanya (1988) analyzed 10 cowpea varieties and observed a range of protein content from 21.5 to 27%, for iron from 8 to 15 mg/100g dry seeds. Nout (1996) evaluated five newly released cowpea varieties for a popular

snack food, “koose” (also called Akara and Kosai in Nigeria). They found that Akara prepared from high-yielding new cowpea varieties Ayiyi (IT83S-728–13) and Bengpla (IT83S-818) were the best. Similarly, Singh (1999b), in collaboration with the Women in Agriculture (WIA) section of the Kano Agricultural and Rural Development Authority KNARDA (Nigeria), evaluated three improved cowpea varieties, IT98D-867–11, IT89KD-288, and IT90K-277–2, and one local variety, Danlla, for four popular local dishes—kosai, danwake, alale, and dafaduka. The dishes were subjected to an independent taste panel of more than 50 people of different economic status and backgrounds. The improved variety IT90K-277–2 was rated as the best, and others were as good as the local variety. None of the varieties were rated as unacceptable. T90K-277–2 has already become very popular in Nigeria and Cameroon as a high-yielding variety. These observations indicate that high yield is not negatively correlated with improved nutritional and food quality traits and that sufficient genetic variability exists to improve these traits in cowpea.

5.9.20 Breeding Cowpeas for Different Cropping Systems

Even though sole crop cowpea is quite profitable, most subsistence farmers plant cowpea as an intercrop involving 1 cereal: 1 cowpea row arrangement,. because land is limited and they want sufficient cereals for home consumption (Singh and Ajeigbe, 2002). The cereals are planted at the onset of rains, and cowpeas are planted 3 to 4 weeks later between cereal rows when rains have stabilized. Thus, cowpea suffers due to shade from cereals throughout the growing period, and the overall grain as well as fodder productivity of cowpea is drastically reduced (Terao et al., 1997). Moreover, cereals as well as cowpeas are planted at very low density, which further reduces total productivity of the system. Therefore, experiments are in progress to study the effect of modifica-tions in the planting pattern, plant densities, date of planting, and row arrangements on grain and fodder yields of the component crops in various intercropping systems.

Six cowpea varieties were evaluated under 1:1 and 2:4 cereals: cowpea intercropping using an improved variety of sorghum, ICSV-400, and local varieties of sorghum and millet (Figure 5.1C,E, F). The objective was to identify the most productive component cowpea and cereal varieties and planting pattern. The cowpea grain, as well as fodder yields, were much higher in 2 cereals: 4 cowpea rows planting compared to 1:1 planting pattern. The mean cereal yield was higher under 1:1 combination compared to 2:4 combination, but considering the combined economic value of cowpea and cereal yields, the 2:4 combination was much superior because cowpea grain are normally priced two to three times higher than cereals. On-farm participatory evaluation of 2 cereals: 4 cowpea strip cropping was initiated in 1998 with 11 farmers. The new system was up to 300% superior in profitability compared to the traditional 1 cereal: 1 cowpea system (Figure 5.1F). This has led to rapid adoption of this system and more donor support (USAID, GATSBY Foundation, DFID, and DANIDA) for rapid dissemination of this technology. Currently, a total of 936 primary farmers are participating in four states of Nigeria, in addition to several thousand secondary and tertiary farmers who have adopted the system on their own. Because of the increased grain and fodder production of cowpea, this system has permitted keeping of livestock on the compound and collecting the manure

for sustaining soil fertility. This is leading to rapid crop-livestock integration (Ajeigbe et al., 2001; Kristjanson et al., 2001; Okike et al., 2002).

5.9.20.1 Cotton—Cowpea Intercropping

Cotton is an important crop in many countries in Sub-Saharan Africa, and it requires frequent sprays of insecticide. The initial growth of cotton is very slow, and it takes more than 150 days to mature. Therefore, it is possible to grow a crop of early-maturing cowpea (60 to 70 days) as an intercrop with cotton. The initial slow growth of cotton does not cause competition, and the insecticide applied on cotton protects the cowpea also. The evaluation of different planting patterns indicated 2 rows of cotton: 2 rows cowpea as the best combination (Table 5.15). The sole crop cotton and cowpea yields were higher than the 2:2 intercropped yields, but when viewed from the land equivalent ratios, the total intercropped yields were significantly higher than the sole crops. For example, even though cowpea and cotton occupied 50% area each in the 2:2 intercrop, their relative yields for both were higher than 50% for all the cowpea varieties and cotton combination

Table 5.15 Performance of Improved Cowpea Varieties under 2 Cotton: 2 Cowpea Intercropping, 2001

	Yield kg/ha under Intercrop				Yield Sole Crop	
Cowpea Variety	Cowpea Grain	Cotton	LER	Cowpea Fodder	Cowpea Grain	Cotton
			Samaru (Nigeria)			
IT95K-193–12	922	1213	1.25	880	1524	1872
IT95K-52–34	784	1269	1.27	924	1300	1872
IT95K-1 072–57	765	1239	1.19	1094	1446	1872
IAR-48 (Local)	407	1252	1.17	856	879	1872
SED Cowpea Grain=48; Cotton=112						
			Maroua (Cameroon)			
IT95K-193–12	720	1129	1.28	488	1239	1600
IT95K-52–34	660	1137	1.28	707	1158	1600
IT95K- 1072–57	588	1139	1.22	1181	1139	1600
24–130 (Local)	636	1075	1.23	892	1934	1600
SED Cowpea Grain=61; Cotton=75						

Table 5.16 Performance of Cowpea under Triple Cropping System (Wheat-Cowpea-Rice)

	Date of Yield kg/ha		
Crop	**Planted**	**Harvest**	**Grain**
Wheat	12/11/98	15/03/99	3229
Cowpea	24/03/99	16/06/99	956
Rice	15/07/99	08/11/99	4483
Wheat	17/11/99	09/03/00	2068
Cowpea	20/03/00	07/06/00	1580
Rice	17/07/00	02/11/00	4500
Wheat	13/11/00	13/03/01	2204
Cowpea	28/3/01	12/06/01	1108
Rice	18/07/01	05/11/01	3509*

* Relied on rainfall, irrigation channel under renovation.

at both Samaru and Maroua. The LER values ranged from 1.17 to 1.27 at Samaru and from 1.22 to 1.28 at Maroua. Considering the higher sale prices of cowpea (at least double) compared to cotton, the 2:2 intercropped gives a much higher income than the sole crop cotton.

Also, cowpea contributes toward enhancing the fertility of the soils. These results suggest that cowpea and cotton intercropping would lead to a sustainable increase in farmers' income in the cotton-growing regions of West Africa.

5.9.20.2 Possibility of Triple Cropping Using Early Cowpea in Rice-Wheat System

Several countries in West Africa have developed irrigation facilities where rice is grown in the rainy season and wheat, cowpea, and vegetables are grown during the dry season. However, most of the farmers grow rice from July to October and wheat from November to March, and the field is normally vacant from April to June. The rice-wheat rotation requires a lot of fertilizer, and it might not be sustainable over time because it does not have a legume in the system. Therefore, trials were conducted using heat-tolerant extra-early cowpea varieties to see if a crop of cowpea could be successfully grown during the idle period from April to June, i.e., after the harvest of wheat and before the planting of rice. The experiment was initiated in November 1998, and it was concluded in November 2001, completing three cycles of wheat-cowpea-rice rotation. The results clearly demonstrated that three crops in a sequence of wheat-cowpea-rice can be successfully grown each year using supplementary irrigation for wheat and cowpea, with a total output

of about 8 tons of food/ha comprising about 2 to 3 tons of wheat, 3.5 to 4 tons of rice, and about 1 to 1.5 tons of cowpea (Table 5.16).

The additional cowpea crop in the summer season (April to June) not only provides extra employment, but it also provides nutritious grain and fodder, which fetch higher prices at that time. Including cowpea in systems also makes the rotation more sustainable and productive because it fixes nitrogen and leaves nutritious residues in the field.

5.9.21 Development and Release of Cowpea Varieties

5.9.21.1 IITA Cowpea International Trials and Variety Release by National Programs

The top-performing breeding lines from the advanced trials are multiplied, and grouped into seven different cowpea international trials (CITs) and distributed to various national programs. CIT-1 has extra-early maturity lines, CIT-2 has medium-maturity lines, CIT-3 has Striga-resistant lines, CIT-4 has insect-resistant lines (no spray), CIT-5 has dual-purpose lines, CIT-6 has vegetable-type lines, and CIT-7 has virus-resistant lines. After the trials have been formulated, a circular is sent to the collaborators in 68 countries informing them about the nature and composition of the trials. According to their requirements, individual collaborators request one or more sets of cowpea international trials. IITA then provides these sets free to the requesting collaborators along with the experimental field books containing the detailed protocols. Based on their results, the scientists in national programs select the best lines from the cowpea international trials and formulate their own multilocational national trials in the following years. The most promising varieties are then multiplied by them and are either released to farmers or used as breeding stock. The collaborative interactions between the IITA cowpea breeding program and the national program scientists have been very effective. A total of 68 countries have identified and released improved cowpea varieties from IITA for general cultivation. The list of countries and the name of breeding lines released are presented in Table 5.17. Many countries where new cowpea varieties are making a difference have given specific names to the new varieties, and, in some areas, farmers themselves have given names and facilitated farmer-to-farmer diffusion of seeds. A few examples are ‘Big Buff’ in Australia; ‘BR-1’ in Cameroon; ‘Titan’ and ‘Cubinata’ in Cuba; ‘Asontem’ and ‘Bengpla’ in Ghana; ‘Akash’ (sky) and ‘Prakash’ (light) in Nepal; ‘Sosokoyo’ in Gambia; ‘Pkoko Togboi’ in Guinea Conakry; ‘Korobalen’ and ‘Sangaraka’ in Mali; Dan IITA (son of IITA) and ‘Dan Bunkure’ in Nigeria; ‘Pannar 31’ in South Africa; ‘Vuli-1’ in Tanzania; ‘Dahal Elgoz’ (gold from the sand) in Sudan; ‘Umtilane’ in Swaziland; and ‘Bubebe’ in Zambia.

5.9.21.2 Cowpea Varieties Released from Other Programs

In addition to varieties released from IITA, a few national programs have also developed cowpea varieties suitable for local adaptation. The U.S. Vegetable Laboratory in Charleston, South Carolina, has released several cowpea cultivars in the past five years. These include the ‘snap’ cultivar ‘Bettersnap’ (Fery and Dukes, 1995b), the cream-type cultivar ‘Tender Cream’ (Fery and Dukes, 1996), and the persistent-green cultivars

'Charleston Greenpack' (Fery, 1998) and 'Petite-N-Green' (Fery, 1999). The persistent-green varieties are an important new market class of cowpea for the freezing industry in the U.S. because they are virtually identical in appearance to fresh-shelled cowpeas after they are imbibed with water. But the harvesting costs are much lower, because persistent-green grains can be harvested dry, with fast, efficient combines, and cleaned and stored dry. With the appearance of a freshly harvested vegetable product, low product cost, and ease of storage and handing, the persistent-green cowpea is attractive to vegetable processors for use in new products or blends with other vegetables. This could help increase cowpea consumption in the U.S. and elsewhere. 'California Blackeye No. 27' (CB27) is a new blackeye cowpea cultivar for production of dry grain that was released by the University of California, Riverside, in 1999. CB27 has high yield, heat tolerance, strong broad-based resistance to root knot nematodes, resistance to two races of Fusarium wilt, excellent canning quality, and a brighter white seed, compared to the standard blackeye variety in California, CB46 (Ehlers et al., 2000b).

Brazil has released 18 varieties in the last 12 years for the northern region. Two of these, Monteiro (Freire Filho et al., 1998) and Riso do Ano (Fernandes et al., 1990a) were obtained through collection and selection in local populations. Sixteen varieties were developed using pedigree breeding. Dry grain yields during the rainy season typically range from 1000 to 1200 kg/ha, while the production under irrigation during the dry season is from 1500 to 2000 kg/ha. These varieties were selected under the rainfed system. Therefore, it is possible that varieties can be developed with much higher yields under irrigation if selection is conducted under these conditions.

Several other varieties have been released in different countries such as 'Charodi-1' (Sreekumar et al., 1993) and 'Vamban 1' (IT85F-2020) (Viswanathan et al., 1997) in India; 'Big Buff' (IT82E-18 Imrie, 1995) and 'Ebony PR' (ADTA, 1996) in Australia; IT83S-852 and IT82D-889 (Lee et al.,1996) in South Korea; Melakh and Mouride (Cisse et al., 1997) in Senegal; IT87D-611–3 (Singh et al., 2002) in Guyana; Cream 7 (Hassan, 1996) in Egypt; IT90K-76, IT90K-277–2, and IT90K-82–2 in Nigeria; Sangaraka (IT89KD-374–57) and Korobalen (IT89KD-245) in Mali; INIFAT 93

Table 5.17 List of Different Countries That Have Released IITA-Developed Improved Cowpea Varieties

Country	Variety Released	Country	Variety Released
Angola	TVx 3236	**Argentina**	IT82D-716
Australia	IT82E-18 (Big Buff)	**Belize**	VITA-3, IT82D-889. IT82E-18
Benin Republic	VITA-4, VITA-5, IT81D-1137, IT84S-2246–4	**Bolivia**	IT82D-889, IT83D-442
Botswana	ER-7, TVx 3236	**Brazil**	VITA-3, VITA-6, VITA-7, TVx 1836–01J
Burkina Faso	TVx 3236, VITA-7 (KN-1)	**Burma**	VITA-4 (Yezin-1)
Cameroon	IT81D-985 (BR1), IT81D-994	**Central African**	VITA-1, VITA-4, VITA-7,

	(BR2), TVx 3236, IT88D-363 (GLM-92), IT90K-277–2 (GLM-93)	**Republic**	VITA-5 TVx 1948–01F, IT81D-1137. IT83S-818. IT82E-18, IT81D-994
		Colombia	IT83S-841
Costa Rica	VITA-1. VITA-3. VITA-6	**Cote D'lvoire**	IT88D-361. IT88D-363
	VITA-7		
Cuba	IT84D-449 (Titan)	**Cyprus**	IT81D-1137. IT85D-3577
	IT84D-666 (Cubinata-666)		
	IT86D-314 (Mulatina-314)	**El Salvador**	TVx 1836–013J (Castilla deseda), VITA-3 (TECPAN V-3), VITA-5 (TECPAN v-5)
	IT86D-368, (IITA-Precoz)		
	IT86D-782 (Tropico-782)		
	IT86D-792 (Yarey-792)	**Equador**	VITA-3
	IT88S-574–3 (OR 574–3)		
Equatorial Guinea	IT87D-885	**Ethiopia**	TVx 1977–01D, IT82E-16. IT82E-32
Egypt	TVu 21, IT82D-716	**Fiji**	VITA-1, VITA-3
	IT82D-709, IT82D-812. IT82E-16		
		Gambia	IT84S-2049 (Sosokoyo) IT83S-728–13
Ghana	IT82E-16 (Asontem)	**Guinea**	IT81D-879, IT83D-340–5, IT83S-818 (Bengpla)
	IT83S-728–13 (Ayiyi)		
	TVx 1843–1C (Boafa)	**Konakry**	IT82E-16, IT85F-867–5 (Pkoku Togboi)
	IT85F-2805, IT83S-990, TVx 2724–01 F (Soronko)		
			IT87S-1463, IT84S-2246–4
Guinea	IT82E-9, IT82D-889	**Guyana**	ER-7, TVx 2907–02D, TVx 66-2H, VITA-3
Bisau			
Guatemala	VITA-3	**Haiti**	VITA-4, IT87D-885
India	VITA-4, TVx 1502	**Jamaica**	VITA-3, ER-7, IT84S-2246–4. IT82E-124

Lesotho	IT82E-889, IT87D-885 IT82E-16. IT82E-32	**Liberia**	IT82D-889, TVx 3236, VITA-5, VITa-4, VITA-7
Malawi	IT82D-889. IT82E-16 IT82E-25	**Mali**	Tvx 3236, IT89KD-374 (Korobalen) IT89KD-245 (Sangaraka)
		Mozambique	IT82D-812, IT83S-18, IT85F 2020
Mauritius	TVx 3236		
Namibia	IT81D-985, IT89KD-245–1, IT87D-453–2	**Nicaragua**	VITA-3
Nepal	IT82D-752 (Aakash) IT82D-889 (Prakash)	**Nigeria**	TVx 3236, IT81D-994, IT86D 719, IT88D-867–11. IT89KD 349
Niger	IT89KD-374. IT90K-372–1-2		IT86D-721, IT88D-867–11, IT82E-60, IT89KD-374, IT90K 277–2, IT90K-82–2, IT89KD 288
Pakistan	VITA-4	**Paraguay**	IT86D-1010, IT87D-378–4, IT87D-697–2, IT87D-2075
Panama	VITA-3	**Philippines**	IT82D-889
Peru	VITA-7	**Senegal**	TVx 3236

Country	Variety Released	Country	Variety Released
Sierra Leone	TVx 1990–01E, IT86D-721, IT86D-719, IT86D-1010, IT82E-32, TVx 3236, TVu 1990, VITA-3	**Somaila**	TVx 1502, IT82D-889 IT82E-32
South Yemen	VITA-5, VITA-7	**South Korea**	VITA-5
		Sudan	IT84S-2163 (Daha ElGoz= Gold from sand)

South Africa	IT90K-59, IT82E-16 (Pannar 311)	**Swaziland**	IT82D-889 (Umtilane), IT82E-18, IT82E-27, IT82E-71
Sri Lanka	IT82D-789 (Wijaya) IT82D-889 (Waruni) TVx 309–01e.g., VITA-4 TVx 930–01B (Lita)	**Thailand**	VITA-3, IT82D-889
		Uganda	TVx 3236, IT82E-60
Suriname	IT82D-889. IT82D-789	**US**	IT84S-2246–4, IT84S-2049 (for nematode resistance)
Tanzania	TKx 9–11D (Tumaini) TVx 1948–01F (Fahari) IT82D-889 (Vuli-1) IT85F-2020	**Yemen**	TVx 3236, IT82D-789, VITA-5
		Venezuela	VITA-3, IT81D-795. IT82D-504–4. TVx 1850–01E
Togo	VITA-5, TVx 3236, IT81D-985 (VITOCO)	**Zambia**	TVx 456–01F, TVx 309–01G, IT82E-16 (Bubebe)
Zaire	VITA-6, VITA-7 IT89KD-349. IT89KD-389, IT89KD-355	**Zimbabwe**	IT82D-889

(Diaz et al.,1997) in Cuba; and GLM 93 (IT90K-277–2) in Cameroon. This is not an exhaustive list, as the information from all the countries is not available.

The availability of high-yielding disease- and insect-resistant varieties with desired seed and growth types is quietly catalyzing rapid increase in cowpea cultivation, including its extension in nontraditional areas. As a result, the world cowpea production has increased from about 1.1 million tons in 1981 to more than 4.5 million tons in 2003.

5.9.21.3 Farmer-to-Farmer Diffusion of Improved Cowpea Seeds in Nigeria

Multiplication and distribution of improved seeds is a major constraint to the rapid adoption of improved crop varieties. The formal seed systems in most countries in Africa are not well established. Even the ones that are in operation concentrate mainly on maize hybrids and other cash crops. Farmer-to-farmer diffusion is considered as a possible option to introduce improved cowpea materials (Singh et al., 1997b).

A project was initiated in 1997 by IITA and the Kano Agricultural and Rural Development Authority (KNARDA), with partial assistance from GTZ, to promote farmer production and distribution of improved seeds. Each selected farmer was given 3 kg breeder seed of the improved cowpea cultivar IT90K-277–2, on credit to be recovered after harvest. Following farmer selection of improved cowpea materials, IT90K-277–2 had been identified as one of the most promising new varieties. A total of 36 farmers

(primary farmers) participated in the project in 1997 and produced 6,786 kg of seed. They sold most of the seeds to 262 farmers (secondary farmers) who had approached them on their own. This group of farmers in turn sold seeds of the improved varieties to the "tertiary farmers." The project has continued, and each year the primary farmers are provided anew with breeders' seeds produced by IITA to ensure that the varietal purity is maintained. The details are presented in Table 5.18.

During the 2001 crop season, 140 farmers participated in the program. They were given 5 kg of seed each of IT90K-277–2. The number of secondary farmers increased to 8,758 and several

Table 5.18 Farmer-to-Farmer Production and Distribution of Seed of the Improved Cowpea Variety IT90K-277–2

Year	Primary Farmers (no.)	Seed Produced (kg)	Secondary Farmers (no.)	Seed Produced (kg)	Seed Produced by Tertiary Farmers (kg)	Total Seed Produced (kg)
1997	36	6,786	–	–	6,786	
1998	51	6,224	262	11,800		18,024
1999	48	18,347	2,458	16,375	64,757	99,479
2000	100	46,250	6,916	173,133	34,847	254,240
2001	140	52,320	8,758	175,160	57,660	285,140
2002	140	238,238	27,375	351,235	75,456	665,012

thousand tertiary farmers. The 140 farmers in the program produced 52,320 kg of seed while the 8,758 secondary farmers produced 175,160 kg of seed. And tertiary farmers produced 57,660 kg of seed to make a total of more than 285 tons. The primary number of farmers was still kept at 140 in 2002, but the secondary and tertiary farmers increased to more than 27,000 and produced more than 600 tons of improved seeds. This has become a very powerful method of disseminating improved seeds of self-pollinated crops in which many seed companies are not interested.

5.10 LOOKING AHEAD

The primary breeding objectives in the past have been to develop a range of cowpea cultivars with combined resistance to major biotic and abiotic stresses to ensure yield stability in sole crop as well as intercrop. Efforts are now being made to breed for higher yield potential, insect resistance (Maruca pod borer, thrips, and bruchids) and higher protein content, and other nutritional traits. Special efforts are being made by IITA and Network for Genetic Improvement of Cowpea for Africa (NGICA) to transfer 'Bt gene' to cowpea for controlling Maruca pod borer for which good sources of resistance are not

available. Research on developing efficient and sustainable cowpeabased cropping systems with primary focus on increased food and fodder production for rapid croplivestock integration is a major priority at IITA. Strategic research in cowpea IPM, in collaboration with national programs, is also continuing to develop new approaches in biological control and habitat management—to complement the efforts in both conventional and biotechnological host plant resistance presented above.

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CHAPTER 6
Faba bean (*Vicia faba* L.)

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6.1 INTRODUCTION

Faba bean is an old crop that has been very well known since ancient times. It was probably domesticated in the sixth millennium BC, that is, in a second wave of domestications in the Near East. It spread quickly in all directions, being taken along by colonizers because of its high protein content and as an excellent crop to alternate with cereals, especially wheat and barley. It was eaten by humans and given to animals. For unknown reasons, although the *favism* can be one of the causes, it had a certain religious role in Egypt, and was forbidden by priests—or as a priestly food—but was appreciated by the people. In Rome, where the *Fabaria* was an important religious festival, old Romans believed in a certain connection between the faba bean seed and the spirits of the dead. In addition, the *Fabii* were one of the noblest families of the ancient Rome. It is not surprising that, being an old crop, it had received many names according to the use given to its products. In English, the common name was *bean*, referring to the typical faba bean seed shape. There were many varieties (landraces), many seed shapes, and many uses. Thus, English farmers talked about *tick beans, horse beans, field beans, broad beans,* etc. In Spain, faba bean is known as *caballares* (for horse feeding), *cochineras* (for pig feeding), and as *habines* (small-seeded varieties) and *habas* (large-seeded ones). *Vicia faba* also gave its name to other crops, especially, as in England, to certain types of *Phaseolus* and *Vigna* species (*fabes, habichuelas,* etc.). In French, the separation of *févèrole* (small-seeded races for animal feeding) *and féve* (for horticultural use and human consumption) is a classical example. In fact, the *name faba bean* is very recent, created by Canadian breeders and spread by the International Center for Agricultural Research in the Dry Areas (ICARDA). In spite of its youth, it is largely used nowadays as a way to simplify so many synonymies for the same species. We gain in simplicity but lose in precision. *Thus, faba bean* will be the name used in this chapter.

6.2 AREA AND PRODUCTION

The cultivated area in the world in 2003 was 2.6 million ha (Mha) dry seeds, with a total production of about 4.1 million metric tons (Mt), The leading country is China, with 1.2 Mha and 1.8 Mt, followed by Ethiopia (0370 Mha, 0.447 Mt), Australia (0.157 Mha, 0.270 Mt), Morocco (0.154 Mha, 0.085 Mt), and Egypt (0.140 Mha, 0.440 Mt) (FAOSTAT, 2004). 180,000 ha were dedicated to horticultural use (green pods and seeds), with a total production already surpassing 1 Mt, with even more striking differences among countries than for dry seed production, as shown by the leading countries (data are given in thousands of ha—mha—and metric tons—mt—respectively): Bolivia (33 mha, 59 mt), Algeria (20 mha, 125 mt), Peru (13 mha, 66 mt), China (12 mha, 118 mt), Morocco (8.5 mha, 103 mt), and Spain (6.8 mha, 61 mt) (FAOSTAT, 2004).

6.3 BOTANY

The genus *Vicia* belongs to the tribe Vicieae. *Vicia faba* L. is the species that gives its name to the Leguminosae family (i.e., Fabaceae), in spite of the lack of a *Faba* genus—although it did once exist: the old Latin taxonomic faba bean name was *Faba bona*. Muratova (1931) defined two subspecies within *Vicia faba* according to the maximum number of leaflets per leaf: ssp. *faba* (*eufaba* in Muratova's terminology) and ssp. *paucijuga*, with respectively more and less than four leaflets per leaf. The latter is a rare form, now probably extinct as a cultivated form, with tiny branches, 1 to 2 sessile flowers per node, small leaflets, and very small, almost rounded, seeds (less than 0.20 g/seed), collected in northwest India and some places in Pakistan.

Muratova (1931) split ssp. *faba* into three botanical types called *varieties* according to the thickness-length seed ratios. A coefficient strongly correlated with the seed size, shape, and weight: *minor* (ellipsoidal seeds up to 0.6 g/seed), *equina* (flattened seeds between 0.6 and 1.1 g/seed), and *major* (very flattened and large seeds with up to 2 g/seed and more). Hanelt (1972) only recognized two subspecies, *faba* and *minor*, including *paucijuga*, in the latter.

Muratova's and Hanelt's names are, then, simple practical groups. They are very useful, as, with a single word, they identify a set of interesting characteristics for agronomists and breeders, but have no other biological value (Cubero, 1984a). Seed and shape of both seeds and pods are the main characteristics defining these groups (Cubero and Suso, 1981; Suso and Cubero, 1986a, 1986b).

6.4 ORIGIN, DOMESTICATION, AND DISPERSION

Very little is known about the origin of *V. faba*. The wild ancestor has not yet been found. It does not cross with any other taxonomic relative, in spite of the many attempts to widen its genetic base (Cubero, 1982a; Cubero, 1984a; Maxted et al., 1991).

Many studies have shown that all forms cross readily to each other; following the Harlan and De Wet (1971) proposal for the taxonomy of cultivated species, *V. faba* should include only one single subspecies, *V. faba faba,* as there is no known wild ancestor. As crosses with other *Vicieae* species have proved impossible until now, only *V. faba* possesses a primary gene pool composed of all the cultivated forms (Cubero, 1982a).

Type *paucijuga* may indeed be the most related form to the wild unknown ancestor. In agreement with studies at the morphological level, it has been shown that no genetic divergence at the isozyme level took place during the dispersion process of the species, except when geographical isolation occurred (Serradilla et al., 1993).

As a crop, it has been cultivated since the late Neolithic Era in the Near East, the most likely place for domestication (Cubero, 1973, 1974; Zohary, 1977). The crop later followed human migrations through different routes. One of them, the European route, crossed Anatolia, Greece, following the Danube Valley from its Black Sea end, reaching Central Europe and then the rest of the continent. A second route toward the west followed the African Mediterranean coast until the Mediterranean west (Maghreb and the Iberian Peninsula), where it could be present by the fifth millennium BC. A third route extended from lower Egypt and Mesopotamia heading southward, reaching Abyssinia. Faba bean in India could have been reached from Mesopotamia or from Abyssinia through the Sabean route, finally, from the Near East, crossing the Caucasus and reaching the Eurasiatic plains (Cubero, 1974). China was probably reached during the first millennium AD, as the Chinese *faba* landraces are only of *major* type, the latest to be produced, likely during the Low Roman Empire. The Spaniards introduced the crop into America in the 16th century. The crop is now also grown in Australia.

The first domesticated types had very small, almost round seeds, much like the *paucijuga* type; there are no archaeological remains of pods, stems, leaves, or any other organ. The *minor* type, which also had small rounded seeds, migrated eastward, while in the Near East, a new shape was probably selected, likely by automatic selection, and was more flattened, similar to our *equina* type. Selection proceeded to increase the flattening of the seed, the only way to reach a considerable size such as that found in the current *major* type (up to 2 g/seed, incompatible, within the pod, with an almost round seed) (Cubero, 1973, 1974). The genetics of primitive characters shows a cluster of reproductive features involving *paucijuga-like* seeds and pods being dominant over their modern counterparts, although the opposite holds true for leaf characters (Cubero and Suso, 1981; Suso and Cubero, 1986a, 1986b; Suso et al., 1984, 1986).

6.5 BASIC FEATURES

Vicia faba is an annual diploid with 2n=12 chromosomes. The root is strong and pivotant. Stems have a square section, are hollow, glabrous, more or less erect (depending on the varietal type), usually branched from the base of the plant, and leaves are without tendrils, composite, alternate, pinnate; the number of leaflets varies according to the varietal group. Leaflet margins are straight or slightly undulated. Stipules show a dark spot with a nectary.

Flowers, typically papilionatae, have a single ovary, usually with 3 to 6 (in some landraces up to 12) seminal rudiments ("ovules") and (9)+1 stamens. In the bottom of the corolla tube there are also nectaries. Flowers are grouped in short racemes (Figure 6.1), with a number of flowers that mainly depends on the varietal type and the geographical origin: *paucijuga* only has 1 to 2 almost sessile flowers; *minor*, especially the spring types, can have 10 to 12 and even exceptionally more; and *equina* and *major* types generally have 4 to 6. Not all of them reach maturity; the number of pods per node depends on both the genotype and the possibility of selfing or outcrossing.

Pods are the typical "legume" fruit that gives its name to the whole Leguminosae family. One to four pods, erect or decumbent, are produced per node in the middle part of the stem. The number of flowers largely exceeds that of pods, this being one of the main breeding objectives.

Pods are internally recovered when they are still green with a soft, velvet-like tissue that is more apparent in *major* forms. Pods of most landraces and cultivars contain an internal layer that contracts at maturity, producing the sudden opening of the two halves of the pod, i.e., the dehiscence. However, some major types have very fleshy pods lacking that mechanical layer, so are indehiscent. They can be cooked and eaten in a similar way to some varieties of peas and common beans. All these forms were undoubtedly selected for horticultural use in rather refined cooking. At maturity, pods are brownish and coriaceous—from cylindrical (*minor* types) to flattened (*major* forms, but also the few *paucijuga*). Depending on the variety group and on cultural conditions, the pod length can vary from very small (2 to 3 cm in *paucijuga*) to very long (30 to 40 cm, and rarely, even 50 cm in some *major* landraces).

The number of seeds per pod ranges between 2 and 10–12. The shape and size of the seeds—from small and almost round or cylindrical to large, flattened, and oval—depend on the botanical group they belong to, Seeds, especially those belonging to the *minor* and *paucijuga* groups, show dormancy that can be broken by treating seeds at 10°C for 3 days, and raising the temperature afterward to 20°C (Mateo-Box, 1961).

Faba bean was a model crop for studies in cytogenetics (Chapman, 1983). Tetraploidy as well as aneuploid (trisomic) lines are known. Attempts in diploidizing the tetraploidy, although successful, did not provide a commercially interesting cultivar (Martín, 1979; Martín and González, 1981; Martín et al., 1986; Poulsen and Martín, 1977). Trisomics are currently used in gene mapping (Cabrera et al., 1989; Satovic et al., 1996, 1998; Torres et al., 1993, 1998). Both tetraploid and trisomic materials were derived from an asynaptic mutant. Trisomics are routinely obtained in this way in faba bean, except when the critical chromosome is the metacentric, as it only appeared once and its extreme

infertility did not facilitate its being maintained. Tetraploid mutants only appeared twice, and both of them provided rather fertile genotypes. The tetraploid is very similar to the diploid forms, with pollen grains of tetrahedron, not ellipsoidal, shapes. The organs are of similar size to the diploid, and the leaflets have undulated margins.

6.6 REPRODUCTIVE SYSTEM

Vicia faba is a partially allogamous species, with entomophylous pollination that determines, to a large extent, the yielding potential of the crop (Duc et al., 1994; Gates et al., 1983). There are many studies on the allogamy rate in faba bean (Free, 1970; Fyfe and Bailey, 1951; Link et al., 1994; Suso et al., 1998). Bond and Poulsen (1983) accepted an average value of 35% allogamy,

Figure 6.1 Flowers in a standard raceme.

ranging from 4% (practically a selfer) to 84% (practically an outcrosser). This suggests that the average value is clearly not representative. The Mediterranean climate seems to favor high allogamy rates; for example, in Southern Spain, 50% of outcrossing is the most frequent value (Suso et al., 1998).

Faba beans are pollinated by bombicids and ants, wasps, etc. (Hymenoptera). Many works have described several *Bombus* species as pollinators. They are *B. hortorum, B. agrorum, and B. terrestris*. Although the latter, which does not perform the pollination, frequently perforates the base of the calyx to have access to the nectaries situated in the

base of the floral tube (Lawes, 1973; Link et al., 1995), this hole is also used by honeybees (Lawes, 1973). Within genus *Apis*, domestic honeybees (*Apis mellifera* L.) have been mentioned as positive pollinators, with different behavior in different races. More effective as pollinators are solitary bees belonging to several genera *(Anthophora, Synhalonia, Eucera)* (Bond and Poulsen, 1983; Robertson and Cardona, 1986). Recent studies suggest that the principal pollinator depends on the locality. In one of these studies, *Eucera numida* was the responsible agent for 92% of the pollination in Córdoba (southern Spain), while in Rennes (northwestern France), there were several *Bombus* species performing 52% of the visits, followed by domestic honeybees (32%), and solitary bees were responsible for only 9% (Cartujo et al., 1998; Suso et al., 1998).

6.7 GROWING HABIT AND PLANT STRUCTURE

Faba beans are photoperiod sensitive, requiring long days to flower and mature without vernalization needs (Duc, 1997). Seeds do not germinate above 20°C (the optimum temperature is 20°C). Temperatures above 30°C in the period between flowering and early maturity can provoke both flower and immature pod abortions, increasing the pod fiber content, decreasing the quality for human consumption as green pods and seeds. Moderate low temperatures are tolerated, although with yield loss.

The natural plant habit of *V. faba* is the indeterminate one. Its development has been the subject of many studies (Peat, 1983), in particular related to the canopy and flower and pod formation. The determinate habit is also known, as in some other legumes (pea, common bean). In the case of *V. faba*, the mutation was artificially produced by x-rays; the allele *ti*, which is responsible of this growth habit, was introduced by Robertson in the 1980s in *major* genotypes of the Near East within the ICARDA breeding program (Robertson, 1997). Later on, it was introduced in Spanish *major* cultivars and released for cultivation. Its main use is for human consumption, providing an easy mechanical harvesting of young (“baby”) pods (Figure 6.2).

Faba bean prefer clay loam, chalky, well-drained and textured soils, with neutral pH, although they adapt to a rather wide pH interval (6 to 9) as well as to sandy loam soils, especially in humid regions (Domínguez, 1999; Maroto, 1989).

Figure 6.2 (See color insert following page 178) Determinate structure of the infrutescence (a) and determinate cultivar under multiplication (b).

6.8 VARIETAL TYPES AND GERMPLASM

6.8.1 Varietal Types

Given the partial allogamy of this species, existing cultivars are either open pollinated populations, or synthetics in some European countries. French breeders hybridized cultivars that, in spite of their good behavior in experimental trials, were not released (Duc et al., 1984). Except for three cultivars still on registration in Spain; all existing cultivars are of indeterminate habit. Landraces obviously also are of indeterminate habit.

Major type, in all cases, is characterized by large and flattened seeds (originating the English name "broad-beans") and were traditionally used for human consumption because of their higher quality, as they usually have edible pods, softer grains, and a low content of bitter chemical (especially tannins). Some landraces of Mediterranean origin, as the Spanish landrace 'Aguadulce' (frequently written in other countries as 'Aquadulce'), are very sweet and palatable, having originated a great number of horticultural varieties all over Europe because of their very high quality.

Both *equina* and *minor* types were used to feed animals, although certain species no longer exist in developed countries: the former for equids (hence the English name "horse-bean," as its Latin name implies) and the latter for pigs (known in Spanish as "cochineras," i.e., related to pigs). The *paucijuga* types were likely used for human consumption.

In central and northern Europe there are two main types of faba bean—spring and winter, according to their sowing dates. In most continental European regions, and in Canada, winter sowings are exposed to hard winters, hence, spring types have been traditionally selected (cultivars usually belong to the *minor,* and rarely *equine* types). Most other countries use autumn sowings because of milder winters; cultivars and landraces are *major* or *equine,* very rarely *minor* types. In all cases, horticultural cultivars or landraces are *major* faba beans.

6.8.2 Germplasm

Faba bean has orthodox seeds from the point of view of conservation; thus, it can easily be stored at low temperature and low humidity. The problem is the maintenance of germplasm collections because of its allogamy. Three means of maintenance have been proposed and practiced—as populations, as inbred lines obtained from the original populations, and as gene pools (Witcombe and Erskine, 1984). These are not incompatible, but complementary, solutions in a genetic resource conservation program.

Large collections are maintained in some research centers, especially at ICARDA (Aleppo, Syria), N.I.Vavilov Institute (St. Petersburg, Russia), and Gatersleben, Germany. ICARDA is the CGIAR center responsible for faba bean resources. The collection is kept in two parts, one in original populations (ILB accessions: international legume faba bean), the other in inbred lines (BPL accessions: faba bean pure lines), obtained after a continuous selfing within populations until reaching homogeneity within BPLs (Hawtin and Omar, 1980; Robertson, 1985, 1997).

6.9 BREEDING OF FABA BEAN

6.9.1 Objectives

6.9.1.1 Yield

The yield of traditional landraces is very low, in the range of 0.6 to 6.0t/ha worldwide. Modern cultivars can at least triple that figure, depending on the environmental factors that include care of the crop, which is usually poor. Besides the objective of a higher yield, common to most if not all crops, in faba bean, the *yield stability* is as important as yield itself as an objective. There are many causes for low yield, including resistance to pests. But in our case, the partial allogamous nature of these plants is an important factor, as individuals produced in the previous generation both by selfing and by outcrossing coexist in every generation. Thus, populations are formed by individuals showing a certain degree of depression by consanguinity, mixed with others showing a certain degree of heterosis. Partial outcrossed populations are more heterogeneous than those reproducing by either strict selfing or by strict allogamy.

There are several ways to overcome this difficulty (Bond et al., 1994):

1. By selecting for a greater stability: Modern multivariate statistical methods allow for precise evaluations of the GxE component of the phenotypic variance, although it is a cumbersome task to be elaborated in long-duration breeding schemes.
2. By obtaining and releasing hybrid varieties: As mentioned, they have been obtained by French breeders (Duc et al., 1984), with good experimental results, but with no success in their commercialization, because of economic factors. Synthetic varieties can also provide a solution; they will be discussed later on.
3. Breeding for self-fertile varieties (Cubero and Moreno, 1984; Stoddard, 1986): A plant is selffertile (also referred to as “auto-fertile”) when it is able to produce the same amount of seeds under selfing than under outcrossing conditions. Lawes (1973) even tried to convert the crop in a strict selfer, similar to common beans, peas, chickpeas, etc. He observed a large variation for this character within a large collection and was able to select lines with a high degree of auto-fertility. Since then, especially in Mediterranean countries, this has been the favorite way of dealing with varietal genetic instability. A self-fertile variety does not completely remove the existence of plants with depression and with heterosis, although it does reduce the problem.

   To select for auto-fertility, it is necessary to select under insect-proof cages, while, at the same time, evaluating the material under open pollination conditions. The morphological basis of self-fertility, its components, and indexes to select for have been studied and designed (Kambal et al., 1976; Mondragao-Rodrigues, 1994; Torres et al., 1993). Self-fertile cultivars offer the possibility of solving the cultural problem in developed countries of heavy pesticide treatments, as they can produce even in the absence of pollinators (Le Guen et al., 1992). It is convenient to repeat here that a good level of pollination will always be convenient, as faba bean, as an original allogamous plant, still shows a good level of heterosis in reproductive characters. Self-fertility helps in avoiding a loss of yield, but the

yield of a self-fertile cultivar will always be less significant than that of a good open pollinated variety in a place where pollinators are abundant.

4. Breeding for wide adaptation: Link et al. (1996a, 1996b) crossed Central European to Mediterranean materials and tested the progenies in both environments with promising results in order to select a widely adapted gene pool. In any case, the populations obtained out of these crosses constitutes an excellent prebreeding material to widen the genetic base of the crop. Among the most important yield components are the number of pods per plant and the number of pods per area unit (Cubero and Martin, 1981; Kambal, 1969), with the number of seeds per pod and the seed size ranking behind. The negative correlation between yield and protein content common in cereals and some grain legumes does not exist, or it is very low, in faba beans, which usually, but not always, even show a positive correlation (Griffiths and Lawes, 1977, 1978).

6.9.1.2 Nutritional Quality

Faba beans can be used as a grain legume or as a horticultural crop. As a grain legume, it is used for feeding animals because of its high protein content, as well as for human consumption. It is not used in Europe for human consumption, except as roasted and salty snack, but it is a staple in Egypt, Sudan, and Ethiopia. The green pods and seeds are a delicacy in some Mediterranean countries, especially the “baby” seeds (length <12 mm), which can reach a high price in the Spanish market. Raw, semi-matured seeds are also consumed as a snack. Both green seeds and pods can be cooked in several Mediterranean traditional dishes. Green seeds are also marketed canned or frozen. Varietal characteristics differ according to their commercial use. In the past, faba beans were also used as green manure. Being rich in 3, 4-dyhydroxyphenylalanine (L-DOPA), which is used in the treatment of Parkinson’s disease, faba bean has a potential use in pharmaceuticals.

Its main use in the Near East as a permanent companion of both wheat and barley historically derived from its high protein content. The following data are the most

	Percentage in Dry Matter
Protein	(20)25 to 35(40)
Carbohydrates	4 to 5
Starch	40 to 50
Amylose	35 to 45
Sugars	3 to 7
Hemicellulose	5 to 6
Lipids	1 to 2
Crude fiber	5 to 10
Crude lignin	1 to 2
Ash	2 to 5

common values found in several studies (Hill-Cottingham, 1983; Hulse, 1994); extreme values are also given in some of the constituents:

Faba bean has usually 25 to 28% protein content and a high lysine proportion. Certain breeding was successfully devoted to increasing the protein content, and some cultivars with as much as 32 to 35% were released. Up to this amount, it seems that the lysine relative proportion is maintained, and beyond that value, breeders have to be careful not to reduce the lysine content (Cubero, 1984b). The nutritional parameters have been the object of several studies (Bond, 1980; Bressani and Elias, 1988; Eggum, 1980; Summerfield and Roberts, 1985).

As usual in legumes and most plants, several anti-nutritional principles challenge the good quality and lower the biological value of its protein. The most important of these constraints are tannins—and vicine and convicine, two glicosids related to favism (Bjerg et al., 1984; Marquardt, 1982). Tannins reduce the biological value by combining with the protein and avoiding its correct processing in the intestinal tract. There is a high correlation between the tannin content and the cellulose of the coat and certain flower colors (Cabrera and Martín, 1986; Griffiths and Jones, 1977). Favism is a reaction (a strong stomachal hemorrhage) produced in individuals homozygous for a recessive allele after eating faba beans—especially, but not only, green seeds and pods. It is not an allergic reaction. It is endemic in the Mediterranean region, where faba bean has been largely consumed in the past, is still a staple in several countries, and, in other countries, is consumed as a snack. In animals, the effects are important only in laying hens and on the reproductive performance of sows (Eggum, 1980); there are no problems for ruminants. Actually, the consequences are not as serious in actual use, as the legumes are never fed alone, but are mixed with other ingredients (especially cereals), even in subsistence farming.

Processing faba bean for feeding animals does not present major problems (Bond, 1980), and including them in concentrates depends only on market prices and political decisions concerning world trade.

There are genes for low content (better than “zero” content, as they usually appear in the literature) in both cases, and some successful programs have introduced these genes—recessive in both cases—in valuable cultivars (Cubero and Duc, 1995; Duc and Cubero, 1998). In the case of tannins, there are two independent recessive genes, *zt1* and *zt2*, each one lowering the tannin content (the double heterozygote does produce tannins by genic complementation). Both of them produce a white color of the flower that can be used as an excellent morphological marker for selecting low tannin genotypes (Picard, 1976). In most European countries, there are white-flowered cultivars. Selecting against vicine-convicine content is more difficult, although the character is determined by a Mendelian recessive allele. The chemical analysis is not yet as quickly performed as breeding work demands; new cultivars being registered in Spain have been obtained by taking advantage of the linkage (5 cM) of the allele for low vicine-convicine content with the white hilum of the seed. Molecular markers more closely linked to the low vicine-convicine allele have been identified.

6.9.1.3 Other Characters

Mechanical harvesting is a must in modern agriculture; faba beans, as are all indeterminate crops, are at a disadvantage in this area. They were never selected for a determinate habit, as in the case of dry seeds as the final product, but were traditionally manually harvested before full maturity and left on the ground until threshing. In the case of horticultural cropping, green pods and seeds were gathered as long as they continued to be produced by the plant. The cost of this operation is not compatible with the requirements of modern use. The *ti* gene for determinate habit is now being introduced to obtain new cultivars apt for green products to be mechanically harvested in extensive plots, and not in gardens (Nadal et al., 2001).

Lodging is caused by heavy rains or excessive watering, by strong winds, overdevelopment of the plant (for example, for excessive applications of nitrogen), and high plant density. Excessively cloudy weather can also be harmful if maintained for a long time. This is unusual under Mediterranean conditions but not in other regions. If harvesting is by hand, there could be no yield loss, but if it is mechanical, as it always is in a developed agricultural system, losses can be very important. Cultivars with strong stems are well known, especially among spring sowing types, which are frequent in European countries and, in general, in humid zones. Stem thickness could be transferred by crossing to other cultivars, but this transfer is advisable only in principle among varieties of the same group, i.e., spring sowing types, as, if selecting for winter sowing in Mediterranean regions, many undesirable traits are also transferred, such as an excessive foliage and late flowering cycles, whose removal by selection is cumbersome. The new determinate flowering cultivars are a new tool to avoid lodging in regions prone to this constraint. Although the first released cultivars have been selected for green pod harvesting, the character can be easily transferred to any other cultivar by simple backcrossing.

6.9.2 Breeding Methods

The peculiarities of faba bean breeding have been considered by several authors (Bond et al., 1994; Cubero and Moreno, 1984; Duc and Picard, 1981; Lawes, 1973; Lawes et al., 1983; Link et al., 1996a, 1996b; Monti and Frusciante, 1984; Muehlbauer et al., 1988; Muehlbauer and Kaiser, 1994). The presence of partial allogamy is to be considered in any breeding program.

6.9.2.1 Bulk-Based Methods

Bulk selection can be applied if working in developing areas where it is necessary to obtain an improved population in a very short time. A basic requirement for success is a high heritability of the character under selection. Yield is not such a character, but under subsistence conditions, a bulk selection can at least typify the local material while setting out a better breeding program at longer term.

In other conditions, recurrent selection is the best option. The largest advance in genetic advance is obtained by separating the generations of selection (performed under insect-proof cages) and recombination (by allowing the material selected to intercross in isolated plots). It is advisable to select during two consecutive years under insect-proof cages, as, if selection is performed only in one year, some of the selected plants can be hybrids produced in the previous generation, showing hybrid vigor. A second year of selfing will remove this possibility. In this way, selection can be performed on landraces, open pollinated cultivars, or crosses among different materials.

Alternatively, it is possible to self in a continuous way to get self-fertile inbred lines that can be multiplied to constitute a self-fertile *quasi* pure line if it has commercial value, or, better, combined with other lines to form pseudo-synthetics (if synthetic values are not estimated) or true synthetics. The former is the preferred option. Mixtures are prepared by choosing inbred lines with similar agronomic characters that show a good yield per plant under cage, that is, a good level of auto-fertility. The pseudo-synthetic mixture is increased in isolated plots.

A strict pedigree method can be followed under cage, i.e., working as if the species were a true selfer, but the system does not offer advantages over the recurrent method explained and, besides, can be very exigent in space under insect-proof cages.

6.9.2.2 Hybrids and Synthetics

A different approach is to exploit the heterosis present in the species. The two classical solutions are hybrid and synthetic varieties:

(a) We have already mentioned the hybrids. Male sterility in faba beans was discovered by Bond et al. (1964, 1966a, 1966b). They found two types of male sterility—nuclear and cytoplasmic. The former was quickly excluded from the possibility of obtaining *commercial* hybrids due to the absence of good morphological markers to facilitate the removal of fertile plants in the rows sown as females in the commercial process.

The main problems found in the handling of the cytoplasmic sterility were, besides the difficulty in finding good restorer genes, the instability of the cytoplasmic male sterility (with a clear drift toward male fertility in a few generations) and the lack of good maintainers of the sterile lines (Duc, 1984; Duc and Huglo, 1984), although new male sterile cytoplasms were identified (Duc et al., 1984, 1985). The reason for that instability was that the male sterility was produced by mitochondrial RNA virus-like particles, which passed to the next generation in irregular numbers, with a critical number being required for complete sterility (Edwardson et al., 1976; Scalla et al., 1981). This problem was

solved later by French scientists, who set up a method for selecting the critical number of RNA bodies by ELISA tests (Dulieu et al., 1988; Berthaut et al., 1991). Valuable hybrids were produced and tested in the field, but alien circumstances prevented commercial release.

(b) Synthetic varieties present a different difficulty; in their design, estimating the general combining ability of a genotype requires complete outcrossing in order to represent the real ability to produce valuable hybrids when crossed with the rest of the genotypes. For faba beans, as for other partial outcrossers, this basic requirement cannot be met because of the high proportion of zygotes produced by selfing. The general approach, in this case, is given by Link and Ederer (1993) and by Maalouf et al. (2002, 1999, 1998); the latter study includes the partial allogamy rates in the formulation, and, thus, is the most general model, although the practical results are similar to those of Link and Ederer (1993), when parental lines are chosen with similar outcrossing rates. In spite of the technical difficulties, synthetic cultivars remain a feasible possibility for the future.

6.10 DISEASES AND PESTS

Many parasites attack *V. faba*, although only a few are of true economic importance. Genetic resistance is available in most of these cases even though very few resistant cultivars have been released. It is an obvious objective of breeding in the future. On the other hand, there also is the possibility of chemical control with its associated environmental disadvantages. Integrated controls remain the best option (Beniwal and Trapero-Casas, 1994; Weigand et al., 1994).

Resistance to diseases and, to a lesser extent, pests, is, obviously, one of the primary objectives in breeding. The main parasite is different in different regions. Chocolate spot *(Botrytis fabae)* and ascochyta *(Ascochyta fabae)* are widely spread, although they become serious diseases in humid regions, such as Atlantic Europe and the Nile Valley. Rust *(Uromyces viciae-fabae)* is widespread, although erratic in its damage. Broomrapes (especially *Orobanche crenata* and *O. ramosai*) are important parasitic weeds in the Mediterranean region—in dry areas as well as in irrigated lands such as the Nile Valley.

6.10.1 Diseases

6.10.1.1 Fungi

Many fungi have been described, although only a few have real economic importance (Gaunt, 1983; Salt, 1983).

6.10.1.1.1 Rust (Uromyces viciae-fabae (Pers.) Shroet.)

Rusts attack aerial organs, especially leaves and stems, producing typical red-brownish powdery lesions. It is widespread, but is important only in some humid and warm regions. Zineb and copper oxyclorure are recommended in endemic zones. Genetic

resistance is known; a quantitative genetic system and a hypersensitive one controlled by a Mendelian gene was recently discovered (Rubiales, 1996).

6.10.1.1.2 Ascochyta (Ascochyta fabae Speg.)

Leaves, stems, and pods exhibit symptoms. On leaves, lesions are dark brown, circular to elliptic, somewhat sunken in the green tissue. The expansion on the leaf changes to an irregular shape and is grayish in the center, with picnidia (a fruiting body containing spores found in certain fungi) becoming apparent. On the stems, spots are similar to those on the leaves but more elongated (Bernier, 1984). Rotations help in decreasing the importance of the disease. Recommended fungicides are clortalonil+copper oxyclorure, folpet+copper oxyclorure, and folpet+cupricalcium sulphate. There is resistance to this disease, regulated by a quantitative genetic system. Lesions in chocolate spot differ mainly by the presence of picnidia, which, when the leaf is observed against the sun, are visible in ascochyta but not in chocolate spot.

6.10.1.1.3 Chocolate Spot (Botrytis fabae Sardiña)

Although stems, pods, and even flowers can show symptoms of chocolate spot under the right conditions, the most affected tissue is the foliar one. Lesions (similar to those produced by ascochyta, but without visible picnidia) vary from small red-brownish dots to large areas, circular at the beginning with a light coffee-colored center and a red-brownish margin. Under favorable conditions (18 to 20°C and very high humidity) (Bernier, 1984), the disease becomes very aggressive, affecting and devaluating pods for commercial use. It is endemic to coastal Atlantic Europe, the Nile Valley, and some other regions. Captan and folpet are recommended fungicides. Genetic resistance does exist, although its quantitative nature does not make its transfer easy.

6.10.1.1.4 Mildew (Peronospora viciae (Berk.) Caspary)

Symptoms are visible in leaf margins, which later dry out (Maroto, 1989). Crop rotation and destroying crop residuals alleviate the presence of mildew. Chemical control can be performed with captan, folpet, and zineb. No genetic resistance has yet been identified.

6.10.1.2 Viruses and Bacteria

Up to now, neither viruses nor bacteria have been considered major diseases, except locally in some cases (Bos and Makkouk, 1994; Bos et al., 1988; Cockbain, 1980, 1983; Mathur et al., 1988), but once cultivars with genetic resistance are dominant, both—especially viruses—will show up. No bacterial diseases are worth mentioning (Salt, 1983). Among viruses, the most frequent are the broad bean mosaic virus, the pea enation mosaic virus, the bean yellow mosaic virus, the broad bean stain virus, and the broad bean leaf roll virus. Some viruses can be transmitted by seed (e.g., the stain virus), and no therapy is known to remove the pathogen.

6.10.2 Parasitic Weeds

Broomrapes (especially *Orobanche crenata* around the Mediterranean Sea, and both *O. ramosa* and *O. aegypytiaca* in the Near East and Egypt humid zones) are parasitic phanerogams without chlorophyll that attract parasites that can destroy the entire crop. Broomrape is an important disease in many Mediterranean areas and affects not only the yield, but even the possibility of sowing. Many areas have abandoned faba bean cultivation because of this parasite. Resistance is known, but very few cultivars that carry resistance genes have been released.

Technically, parasitic weeds should be considered a disease, but they also share characteristics of standard weeds. In fact, they can be controlled with herbicides. Because of this double aspect, they deserve their own heading. We will consider only the broomrapes, as dodder *(Cuscuta graveolens)* affects crops only under an extreme lack of care in farming.

Orobanche seeds need a stimulant produced by the host to germinate; when the haustorium produced reaches the host root it penetrates the roots, connecting its own vascular system to that of the host, from which it will extract mainly water and sugars. A reddish nodule is formed that develops a powerful shoot, single in *O. crenata*, many in *O. ramosa* and *O. aegyptiaca.* Once it has emerged from the soil, the initial growing rate can be very rapid—as much as 1 cm/day. The shoots have their leaves reduced to scales. The large number of flowers can produce up to half a million seeds per plant. A faba bean crop (as well as many other crops) can be totally destroyed by a strong attack, and farmers of affected areas tend to abandon the crop.

Several reviews have dealt with different aspects of the host-parasite relationships (Cubero, 1982b, 1993; Cubero and Moreno, 1979, 1999; Cubero and Rodríguez, 1999; Cubero et al., 1999). Egyptian breeders selected the resistant line F402, out of which the cultivar ‘Baraca’ was obtained in Spain through a recurrent selection program involving local materials with different degrees of tolerance (Cubero and Moreno, 1999). This resistance, controlled by a genetic quantitative system (a stable QTL has been identified recently), has proven to remain under very different ecological conditions, such as those represented by Andalucía (southern Spain), the Syrian coast at Lattakia, Tunisia, and Morocco. However, it does not resist *O. foetida* in Tunisia, where this new broomrape is currently restricted as a parasite because it is a potential danger for all the Mediterranean areas. It does exist in other regions without being a pest—at least so far. The reasons for this difference in behavior remain unknown.

Glyphosate is active against broomrape at low concentrations (60g a.i./ha). Treatments are more effective at the nodular stage of the parasite, usually simultaneous with the flowering period, which is why it is recommended to perform the treatment at this stage, much easier for farmers but somehow incorrect. The use of a tolerant (not resistant) cultivar, together with glyphosate applications, is also a valid method of control.

6.10.3 Nematodes

Nematodes are serious pests in some regions, although they are also spread in many regions without challenging the yield (Hooper, 1983). Stem nematode (*Ditylenchus dipsaci* Filipjev) is the most important to faba beans, especially in cold regions. Young

stems thicken, and this thickening later affects the adult stem, which becomes reddish-brown at the beginning and ends up almost black. Petioles and leaflets are typically deformed. The infection can pass to the pods and seeds. The parasite can persist in the soil for several years, even without the host presence (Hooper, 1976). To disinfect the seed as a preventive treatment, aldicarb can be used (Bernier, 1984). Symptoms can easily be mistaken for a chocolate spot infestation. If the cuticle of the stem is removed, nematodes occasionally can be seen without the help of a lens.

6.10.4 Pests

We mention here only insect pests, as they are largely the most important ones (Bardner, 1983; Weigand et al., 1994). There is no genetic resistance against them, although some genotypes exhibit low levels of tolerance—not commercially interesting—against some insects.

6.10.4.1 Bruchus (*Bruchus rufimanus* Boheman)

Bruchus is a polyphagous granary pest. The female sets eggs inside the grain at young stages of the pod. The larvae feed inside the seeds, destroying them either when still in the field—or later, after they are harvested. The adults overwinter in the granaries and migrate to the field in the spring. Hence, a complete disinfection of the premises is compulsory to get rid of the plague (Domínguez, 1999). Chemical control can be obtained with deltametrin (2.5%) and deltametrin (2.5%)+heptenofos (40%), applying the treatments before the final flowering stages and the beginning of pod setting.

Resistance to other bruchids has been found in other legumes besides faba bean, such as cowpea *(Vigna unguiculata)* and common bean *(Phaseolus vulgaris)*. In the latter, a gene found in wild populations in Mexico was transferred to cultivated materials by backcrossing. To identify and clone the responsible genes in both species could be an important achievement, as it would permit their transfer via genetic engineering to other legumes such as *Vicia faba*. In faba beans, some lines have been found that produce seeds (always of light color) that remain free as well as some (always dark) that are attacked by the bug, but that resistance has proven impossible to be fixed by selection. Whether that resistance is related to a transposon or any other mobile element remains to be studied. The reduced importance of this bruchid in developed countries because of the availability of chemical control discouraged this research.

6.10.4.2 Sitona (*Sitona lineatus* L.)

Adults of sitona attack the leaves, eating the leaf margins with a set of typical semicircular incisions. Larvae live below the ground, damaging the roots and, especially, the Rhizobium nodules that are either destroyed or diminished in their activity. Insecticides such as deltametrin and heptenofos can be used, as for bruchids.

6.10.4.3 Lixus (*Lixus algirus* L.)

Lixus are borers that grow within the stems, drying out the plant. Deltametrin and heptenofos are recommended again.

6.10.4.4 Aphids (*Aphis fabae* L.)

Aphids are polyphagous, especially *Aphis fabae*, a well-known enemy of many plants (more than 200 hosts have already been mentioned) (Cammell and Way, 1983). They are suckers, living in the uppermost parts of stems and in the buds, deforming the plant shape and stopping its growth if not controlled. If the attack is serious, even the floral racemes can be attacked. Important as the direct damages are, the indirect ones cannot be overlooked. The sugary liquid that aphids excrete is a growth medium for several fungi that cover the organs with a black-colored layer. Aphids are well known transmitters of viruses, such as the broad bean mosaic virus and many others (Bos and Makkouk, 1994). Chemical control is achieved with deltametrin, deltametrin+heptenofos, lambda cihalotrine, and several other active substances.

6.11 NITROGEN FIXATION

Nodulation is produced by *Rhizobium leguminosarum*, common in all faba bean areas, also nodules on many other legumes, both wild and cultivated, with a high efficiency. If the crop is introduced in a new region, artificial inoculation is compulsory. The amount of nitrogen fixation by the nodules varies between 60 and 250 kg/ha/year (López-Bellido and López-Bellido, 2000; Roughley et al., 1983; Summerfield and Roberts, 1985), with some extraordinary data in the literature (up to 600 kg/ha/year) (Sprent and Bradford, 1976). Nitrogen fixation starts 3 to 4 weeks from germination and lasts until leaf senescence. As in most crops, there is an interaction between host and Rhizobium genotypes, which leads to a large specificity rhizobium strain-faba bean line (Griffiths and Lawes, 1977), affecting even the protein content of the host line. There have been several attempts to select for higher efficiency (Beringer et al., 1988; Bliss and Miller, 1988; Herridge et al., 1994), although, unfortunately, there are many drawbacks to using this interaction in a practical way (Stanforth et al., 1994).

6.12 BIOTECHNOLOGY

In most, if not all, cultivated species, many attempts have been made to try to incorporate alien genes in the genome of *Vicia faba*. Before the genetic engineering period, the extra-specific gene transfer was intended by interspecific crosses, and in recent times, by the usual ways in biotechnology.

6.12.1 Interspecific Crosses

The likely candidate sources of useful genes were their closest botanical relatives. *Vicia faba* was usually included in a section, *Faba* (Kupicha, 1976), which also included *V. narbonensis* and its related species, often known collectively as "*narbonensis* complex" because of the many similarities existing among them. *Vicia narbonensis* and its relatives were for a long time the species chosen to widen the gene pool of *V. faba*. (In the past, some botanists preferred *narbonensis* a section including *V. faba*, but that botanical treatment is irrelevant in this discussion).

However, DNA analysis had shown the great difference in DNA content recorded within the genus *Vicia*, with a sixfold variation existing between the smallest (3.85 pg in *V. monantha*) and the largest (27.07 pg in *V. faba*). The DNA difference is twofold between *V. faba* and all the *V. narbonensis* complexes (Chooi, 1971; Raina, 1990). In addition, some works on numerical taxonomy, using morphological characters, have pointed out that the similarity between *V. faba* and *V. narbonensis* and the other species of its section *(V. serratifolia, galileae, johannis, bithynica)* was more superficial than actual (Bueno, 1976; Cubero, 1982a, 1982b), with *V. faba* occupying an isolated place in the dendrogams. Chromosome size, symmetry, and Giemsa banding patterns of *V. faba* and *V. narbonensis* also were completely different (Dobel et al., 1973; Singh and Lelley, 1982).

The conclusion of all these works is that *Vicia faba* is a rather isolated species in the genus *Vicia*, of unknown origin, and whose chromosomic and genomic DNA features remain largely unexplained. In spite of these data—as well as of some previous negative results (Hanelt et al., 1972; van Cruchten, 1974)—the International Center for Agricultural Research in the Dry Areas (ICARDA) requested a study to try to solve the problem. The extensive study included the use of pistil mutilations, mentor pollen, chemical manipulations *in vivo*, embryo rescue, and interspecific grafting without positive results (Pickersgill et al., 1983; Ramsay et al., 1984; Ladizinsky et al., 1988). Embryos can be produced, but fail after 2 weeks—before they are large enough for *in vitro* culture. *V. faba paucijuga* lines were the best parents for interspecific crosses when used as females, perhaps because they are the closest to the elusive wild ancestor (see Section 6.3, Botany) and *V. galilaea* and *V. narbonensis*, respectively, are the best and worst pollen parents in fertilizing faba bean ovules. *V. faba* seems reproductively, as well as taxonomically, isolated within its genus. Thus, currently, the use of extra-specific variation by crossing does not seem feasible.

6.12.2 Genetic Engineering

As in many other crops, extra-specific gene transfer has also been attempted in faba beans since the 1970s, although very few attempts at genetic transformation have been performed. A previous step to transformation is to obtain regenerated plants by *in vitro* techniques, a problem already solved (Sayegh, 1988; Tegeder et al., 1995; Fernandez-Romero et al., 1998). One of the main obstacles was the high mortality when transferring the regenerated shoots to the soil. The best solution, from a practical point of view, was to graft the regenerated shoots onto rootstocks from decapitated young plantlets.

Thus, regeneration is possible, but in spite of some attempts in several laboratories, no one has claimed full success in obtaining economically useful transgenic faba beans—although *Vicia faba* could be transformed by using *Agrobacterium tumefaciens*, as well as by DNA particle accelerator, as in soybeans. Legumes, in general, are hard to transform, and only very valuable crops, as soybeans, for example, support the whole process (i.e., from the laboratory to field trials). Pea, for example, has also been transformed, but commercial transgenic cultivars are not yet available. Success in transforming a legume is an economical problem more than a technical one. In fact, it is not difficult to obtain GUS-transformed tissues of faba bean under *in vitro* culture by DNA accelerator as a common student practice work. As mentioned above, to obtain regenerated plants would not be difficult, but to complete the process in obtaining an adult plant, then to transfer its character by traditional backcross to commercially valuable genotypes, and, finally, to follow the costly and complex system for registration and spreading, particularly in the EU, is not yet feasible for faba beans.

A second reason for the weak effort in obtaining transformed faba beans lies in the character to be transferred:

1. Concerning resistance to *Orobanche*, resistance to glyphosate could be a candidate as, in very low concentration, this herbicide is applied to control broomrape, but there is also now a good source of resistance to this parasitic weed, although its quantitative nature makes its handling in breeding programs cumbersome. Besides, new genetic sources have been identified. Furthermore, determinate growing genotypes tolerate a double rate of the active ingredient than the indeterminate ones. Thus, by choosing adequate genotypes and sowing dates, broomrape can be controlled satisfactorily.

   In other crops, other strategies have been sought, such as transferring genes that control specific toxins whose action in the transgenic roots of the host inhibit *Orobanche* growth. But only preliminary results are known, and further tests are required, because, although a possible result could be a certain action against the parasite, it might not be important enough for commercial use.
2. Resistance to aphids and bruchids would be very convenient, especially for developing countries, but genes for these pests have not yet been identified. A cowpea antitripsic gene, effective against cowpea bruchids, has been identified and cloned; and it could be a good candidate for faba bean, provided that the gene is also effective against its specific bruchid *(Bruchus rufimanus)*.
3. Genes controlling resistance to several diseases have been identified, as previously mentioned, and it is thought that an even greater effort in identifying new valuable sources should precede the transfer of alien genes by genetic engineering, if available. Viruses, in general, are not serious diseases, although in some places and in some years, serious damage was recorded. Some effort in traditional selection to identify sources of resistance by classical means should be carried out before turning to genetic engineering. The production of faba bean genotypes resistant to yellow mosaic virus and faba bean necrotic yellow virus has been attempted, although, to our knowledge, no positive result has been reported.
4. Antinutritional substances such as vicine and convicine (main favism factors) and tannins can be eliminated by traditional backcross programs, as major genes for these factors are known. With both, morphological markers can be used—the white seed

hilum for low vicine-convicine content (placed at a distance of 5 cM) and the white flower color for low tannin content (a pleiotropic

Figure 9.1(a), (b), (c) and (d) Flowers and seeds of: **(a)** *Lupinus albus,* **(b)** *Lupinus luteus,* **(c)** *Lupinus angustifolius* (wild type flower color shown), and **(d)** *Lupinus mutabilis*.

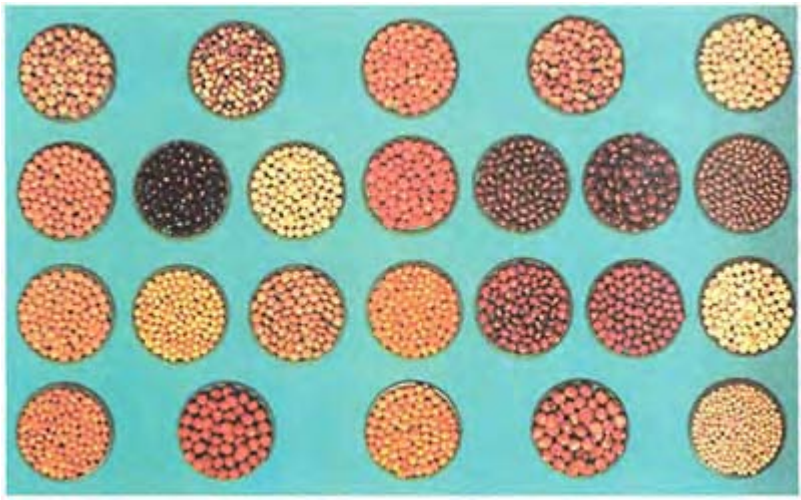

Figure 4.1 Seed color variation in pigeonpea.

a

b

Figure 6.2(a) and (b) Determinate structure of **(a)** the infrutescence and **(b)** determinate cultivar under multiplication (Faba bean).

Figure 7.1 Breeding chickpea for early maturity at ICRISAT. Short-duration varieties have helped adaptation of chickpea in tropical environments.

gene). Obviously, selection can be accelerated by using molecular markers, which can permit the identification of the critical genotypes at a very early stage. Be that as it may, genetic engineering is out of reach.

5. A higher protein quality could be achieved by transferring a gene controlling a sulfur-amino acid rich peptide. Such genes are known, such as in sunflower, and have been transferred to soybean and pea. They could be good candidates for obtaining faba bean genotypes with a higher rate of methionine and cysteine, provided the lysine content of the transgenic line remains high.
6. Transferring resistance or tolerance to glyphosate, ammonium gluphosinate, and bromoxinil herbicides can be interesting and feasible. The case of glyphosate has been mentioned above in this section in connection with resistance to broomrape. Its transference would also help in controlling a wide weed spectrum; the two other herbicides do not have any action against broomrapes, but likely the transgenic genotypes could be useful for controlling weeds, a serious problem in most legumes, although nowadays several good herbicides are available.

To summarize this section, genetic engineering could be attempted in faba beans with a fair probability of success, but, in general, it would be very convenient to intensify the identification of new genetic sources for most characters. However, as in the case of the protein quality by modifying the aminoacidic proportions, there are clear candidates for a biotechnological approach.

6.12.3 Other Biotechnological Achievements

A composite molecular map has been successfully developed that includes morphological markers, isozymes, RAPDs, SCARs, seed protein genes, and microsatellites. By using trisomics, the linkage groups have been placed in their carrying chromosomes (Torres et al., 1995; Vaz-Patto et al., 1998, 1999); for the long metacentric chromosome, whose trisomics could not be obtained, some markers were developed to build up its chart. The linkage groups so far obtained cover about 1600 cM with an overall map interval of 8 cM. Several important characters have been mapped, such as genes and quantitative trait loci (QTLs) for resistance to ascochyta, rust, and broomrape, as well as for the two main antinutritional factors (Roman et al., 2004). In this map, genes controlling important characters, both of a qualitative (Mendelian) and quantitative (QTLs) nature, have been placed. Marker-assisted selection (MAS) as well as studies on syntheny are the ultimate objectives.

6.13 LOOKING AHEAD

Vicia faba has a long history as a cultivated plant. It was spread by Neolithic farmers in all directions from the Near East, and colonized in very old times in North Africa, Europe, and Central Asia. Its use for both feeding animals and for the humans' table produced a large variation in shapes, habits, and tastes. Spread again by Celts (it was for a long time the "Celtic grain"), then by the Romans, it also reached China more or less 2000 years ago, America just after Columbus' voyage, and South America and Australia in very recent times.

The shape of its seeds became *the* shape to describe other seeds: *Vicia faba* was *the bean*. Later, its name was borrowed by other crops and, especially by the *Phaseolus* crops, which, finally, almost entirely appropriated it.

The substitution of horses and oxen by machines was an almost mortal blow to faba beans, as it was to many other legumes. However, their culture was maintained because most farmers were more confident of their reliability compared with other new crops. The best institutions of agronomic research in the 20th century almost forgot them, as was the case for many other legumes.

In the 1970s, a research program was set up by the EU (then the European Economic Community, EEC) to reduce the strong dependence on external sources of protein for animal feeding. At the same time, the Consultative Group on International Agricultural Research (CGIAR) created ICARDA (International Center for Agricultural Research in the Dry Areas) to work on a huge and difficult agricultural and geographical area, and placed faba bean under its mandated crops. The joint work produced a vast amount of information and new genetic variation. New cultivars were obtained that yielded well in developing countries, although the research effort devoted to them was only minimal in comparison with the major cereals. Important multidisciplinary research was performed on them by ICARDA and European researchers, but the gap between such research and that devoted to major food crops is wide and deep.

Many important problems in the cultivation of faba bean have been solved. The big problem with faba bean is that the policy makers never took into consideration the need for a package of improved practices to accompany new varieties. As a result, most farmers still sow faba bean with the *older style* management, that is, only sowing and harvesting. However, to yield well, the modern faba bean cultivars require the same care as any other modern crop including herbicides, pesticides, fertilizers, good seed quality, and so on. Farmers and their advisors should consider the economic and management benefits of applying good farming principles *with modern* cultivars. Feedback by farmers on new practices is lacking, and there is no cycle of continuous research refinement to work with farmers to improve crop management.

New research avenues are clear. New cultivars need different agricultural practices than those used in subsistence farming. Such practices must be tuned to fit every environment by simple but productive research in the classic perpetual cycle of farming-breeding interactions. We have mentioned the use of long-known mutants to build up new cultivars showing either new growing habits or resistance to pests or with better nutritional qualities. We have to better explore the genetic diversity of the species, and to try again to introduce beneficial alien variations from other species (plant explorer, archeobotanist, geneticist, and plant breeder Jack Harlan always said that "*Vicia faba* ancestor has to be some part") by genetic engineering. We also mentioned, in the preceding section, the best candidates to be transferred this way.

Not to be forgotten is faba bean use in sustainable agriculture, as well as in organic or biological farming. The large amount of fixed nitrogen is perhaps the best known characteristic for that purpose, but not to be neglected is the excellent soil structure it leaves. In our region, Andalucía (southern Spain), not too long ago, when an uncultivated field was to be put in cultivation, the first crop was faba bean, then a cereal. Reintroducing faba bean in rotations will have a positive effect, as demonstrated so many times by studying the economic profit of faba bean-cereal/sunflower alternatives for the medium or long term, rather than on a yearly and subsidized basis.

To summarize, there is a place in modern agriculture for faba bean. Some voices claim—rightly—that pea yields more in some regions, chickpea or lentil in others. But the answer is not to just substitute a certain crop with another, but to use all of them. Promoting the use of different legumes is the best way to avoid, or at least delay, the appearance of new pests, and at the same time produce a better use of the soil. A patched countryside, not a uniform one, is perhaps the only way to attain sustainability.

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CHAPTER 7
Chickpea (*Cicer arietinum* L.)

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7.1 INTRODUCTION

Chickpea (*Cicer arietinum* L.), commonly called gram, Bengal gram, or garbanzo bean, is the most important food grain legume of South Asia and the third most important in the world after common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.). Chickpea is a diploid with 2*n*=2*x*=l6 chromosomes and a genome size of approximately 750 Mbp (Arumuganathan and Earle, 1991). Chickpea is one of the first grain crops cultivated by man and has been uncovered in Middle Eastern archaeological sites dated to the eighth millennium BC (Zohary and Hopf, 2000). Two distinct market type classes, *desi* and *kabuli*, are recognized in chickpea (Pundir, Rao, and van der Maesen, 1985). The *desi* types that account for about 85% of chickpea area usually have small, angular-shaped, dark-colored seeds with a rough surface, pink flowers, anthocyanin pigmentation on the stems, and either semi-erect or semi-spreading growth habit. The *kabuli* type, which cover the remaining 15% area, usually have large "rams head"-shaped smooth surface seeds, lack of anthocyanin pigmentation, and semi-spreading growth habit.

It has become increasingly clear during the last few decades that meeting the food needs of the world's growing population depends, to a large extent, on the conservation and use of the world's remaining plant genetic resources. Conservation without use has little point and use will not come without evaluation. Genetic resources encompass all forms of the cultivated species, as well as their related wild species (Harlan, 1984). That is a general concept to which chickpea is no exception. In reviewing genetic resources and their multifaceted applications in chickpea genetic improvement, we have placed more emphasis on the wild genetic resources of the cultivated chickpea, while providing a brief overview of resources available in the cultivated species.

7.1.1 Global Chickpea Production and Distribution

Chickpea is grown in more than 40 countries, but the main growing region is South and Southeastern Asia (ca. 70%), where India (ca. 60%) and Pakistan (ca. 10 to 15%) are major contributors. West Asia accounts for approximately 16% of global chickpea area, with Iran, Turkey, and Syria being the largest producers in the region. Africa accounts for

5%, mostly from Ethiopia, Malawi, and Tanzania in Eastern Africa and Morocco in North Africa. In remaining parts of the world, about 3% area is contributed by North America (mainly Canada), 2% by Australia, and 1% by Europe (mainly Spain).

The global chickpea area has remained almost stagnant during the past 40 years; it was 11.8 m/ha during 1961–1965 and 11.0 m/ha during 1996–2000. Though there has not been much change in the global chickpea area, the production has increased from 7 m/t in 1961–1965 to 8.4 m/t during 1996–2000, due to enhancement in yield levels from 599 to 791 kg/ha^{-1} during this period. During 2000–2002, chickpea production was 7.5 m/t from an area of 9.7 m/ha (average of three years).

There has been more than a sevenfold increase in the global trade of chickpea during the past four decades. Trade increased from 0.13 m/t during 1961–1965 to 0.66 m/t during 1996–2000 and reached its highest level of 1 m/t during 2001. India has been the world's largest chickpea importing country during the past 15 years. It had a record import of 0.52 m/t during 2001. Pakistan has emerged as the second largest importer of chickpea during this period, its imports increasing from 5,000 t during 1981–1985 to 0.1 m/t during 2001. Other countries that have had sizable chickpea import during recent years are Spain, Saudi Arabia, Italy, Jordan, Tunisia, Lebanon, Turkey, Sri Lanka, and Colombia (Gowda and Gaur, 2004).

7.1.2 Importance of the Crop

Chickpea has one of the highest nutritional compositions of any dry edible grain legume and does not contain significant quantities of any specific major antinutritional factors. On an average, chickpea seed contains 23% of highly digestible protein, 64% total carbohydrates, 47% starch, 5% fat (primarily linoleic and oleic acids), 6% crude fiber, 6% soluble sugar, and 3% ash. The mineral component is high in phosphorus (343 mg/100 g), calcium (186 mg/100 g), magnesium (141 mg/100 g), iron (7 mg/100 g), and zinc (3 mg/100 g) (Williams and Singh, 1987). Used in a variety of ways, chickpea is not only good for human health but also for soil health. It meets 80% of its nitrogen (N) requirement from a symbiotic rhizobial interaction, which enables the crop to fix up to 140 kg N ha^{-1} from atmosphere (Saraf et al., 1998). It leaves substantial amount of residual nitrogen behind for subsequent crops and adds much needed organic matter to maintain and improve soil health, long-term fertility, and sustainability of the ecosystems. In recent years, chickpea has also gained popularity in broad-acre cropping systems in developed countries, particularly Australia and Canada (Siddique and Sykes, 1997). A dryland crop requiring minimal inputs, chickpea is a boon to the resource-poor marginal farmers in the tropics.

7.2 ORIGIN OF CROP AND ITS TAXONOMY

7.2.1 Origin of Chickpea

The available evidence suggests that chickpea originated in the fertile crescent region of southeastern Turkey and adjoining Syria (van der Maesen, 1987). The proposed wild progenitor and several other annual *Cicer* species occur there (Ladizinsky, 1975). Further evidence of its origin comes from seeds dated to about 5450 BC, unearthed from archaeological excavations at Hacilar near Burdur in Turkey (Helbaek, 1970).

It is believed that chickpea diverged from Turkey in two directions—into the western parts where it is grown in the spring and summer and into the eastern and southern parts where it is grown in the cool dry seasons. The majority of the wild *Cicer* species are found in the West Asia and North Africa region covering Turkey in the north to Ethiopia in the south, and Pakistan in the east to Morocco in the west. Botanically, the cultivated chickpea has been split into two groupings, *microsperma* and *macrosperma*, corresponding to seed size and in much the same way as it has been done for lentils (Cubero, 1987). From a practical point of view, chickpea is also classified into *kabuli* and *desi* types. The terms *desi* and *kabuli*, however, do not overlap with *microsperma* and *macrosperma*. The *kabuli* types are now grown predominantly in the countries of the Mediterranean region, West Asia, North Africa, Australia, and North America, while the *desi* types are grown mostly in South Asia, Iran, Ethiopia, Mexico, and Australia. Other than morphological differences, all forms of the cultivated species share the same genome.

The wild annual progenitor of chickpea has been repeatedly identified as the annual species *C. reticulatum* Lad. (Ladizinsky and Adler, 1976b; Ahmad, Gaur, and Slinkard, 1992; Iruela et al., 2002). It is believed that one of the crucial steps in the origin of cultivated chickpea was the change from the perennial to annual life cycle (Gupta and Bahl, 1983). The perennial species have not been extensively studied, however, the available evidence suggests *C. anatolicum* Alef. to be the probable perennial progenitor of *C. arietinum* (Gupta and Bahl, 1983; Ahmad, 1989; Tayyar and Waines, 1996). It appears that domestication and, thus, evolution of the cultivated species followed the usual process of artificial selection, which favored large palatable seeds, reduced pod dehiscence, nondormancy, synchronous ripening, earliness, and diversity of forms (van der Maesen, 1987).

7.2.2 Taxonomy of *Cicer*

The cultivated chickpea species has been taxonomically placed in the genus *Cicer*, which belongs to the family Fabaceae and its monogeneric tribe Cicereae Alef. (Kupicha, 1981). Presently, the genus *Cicer* consists of 43 species (Table 7.1), divided into 4 sections, *Monocicer*, *Chamaecicer*, *Polycicer*, and *Acanthocicer*, based on their morphological characteristics, life cycle, and geographical distribution (van der Maesen, 1987; http://singer.cgiar.org/Search/SINGER/search.htm). Eight

Table 7.1 List of All Known Species in the Genus *Cicer*

Annual Species	
*C. arietinum** L.	*C. judaicum** Boiss.
*C. bijugum** K.H.Rech.	*C. pinnatifidum** Jaub. & Sp.
*C. chorassanicum** (Bge.) M.Pop.	*C. reticulatum** Ladiz.
*C. cuneatum** Hochst. ex Rich.	*C. yamashitae** Kitamura
*C. echinospermum** P.H.Davis	
Perennial Species	
C. acanthophyllum Boriss.	*C. macracanthum* M.Pop.
*C. anatolicum** Alef.	*C. microphyllum** Benth.
C. atlanticum Coss. ex Maire	*C. mogoltavicum* (M. Pop.) Koroleva
C. balcaricum Galushko	*C. montbretii** Jaub. & Sp.
C. baldshuanicum (M.Pop.) Lincz.	*C. multijugum* van der Maesen
*C. canariense** Santos Guerra & Lewis	*C. nuristanicum* Kitamura
C. fedtschenkoi Lincz.	*C. oxyodon* Boiss. & Hoh.
C. flexuosum Lipsky	*C. paucijugum* (M. Pop.) Nevski
*C. floribundum** Fenzl.	*C. pungens** Boiss.
C. graecum Orph.	*C. rassuloviae* Lincz.
C. grande (M. Pop.) Korotk.	*C. rechingeri* Podlech
*C. heterophyllum** Contandr et al.	*C. songaricum** Steph. ex. DC.
C. incanum Korotk.	*C. spiroceras* Jaub. & Sp.
*C. incisum** (Willd.) K.Maly	*C. stapfianum* K.H.Rech.
*C. isauricum** P.H.Davis	*C. subaphyllum* Boiss.
C. kermanense Bornm.	*C. tragacanthoides* Jaub. & Sp.
C. korshinskyi Lincz.	
Unspecified	
C. laetum Rassulova & Sharipova	

*Species with confirmed somatic chromosome number of 2n=16
Source: The CGIAR System-Wide Information Network for Genetic Resources (SINGER; http://singer.cgiar.org/Search/SINGER/search.htm) and van der Maesen, 1987. Adapted from Croser et al. 2003a.

of these *Cicer* species, sharing the annual growth habit with chickpea, are of particular interest to breeders. Of the nine annual *Cicer* species, eight are classified within the *Monocicer* section, and one, *C. chorassanicum*, within the *Chamaecicer* section (Kazan and Muehlbauer, 1991; Muehlbauer, Kaiser, and Simon, 1994). Thirty-three of the remainder species are known to be perennial, while *C. laetum* Rass. & Sharip has an unspecified life cycle (van der Maesen, 1987).

7.3 GERMPLASM COLLECTION, MAINTENANCE, AND EVALUATION

Due to its importance as an affordable protein source in developing nations, it is crucial to obtain and maintain higher yields of the chickpea crop. Effective genetic means are, thus, needed to reach such key objectives. The effectiveness of selection in any crop depends upon the extent and nature of phenotypic and genotypic variability present in different traits of the population (Arora, 1991). Unfortunately, the chickpea crop is susceptible to a range of biotic and abiotic stresses, which can be devastating to crop yield. A low level of genetic variability within *C. arietinum* has hampered chickpea breeders in their efforts to develop widely adapted cultivars with resistance to biotic and abiotic stresses (Ladizinsky and Adler, 1975; Tuwafe et al., 1988; Kazan and Muehlbauer, 1991; Ahmad et al., 1992; Ahmad and Slinkard, 1992; Udupa et al., 1993; van Rheenen et al., 1993; Labdi et al.,1996; Tayyar and Waines, 1996; Simon and Muehlbauer, 1997; Siddique et al., 2000). Recently, this low level of genetic variability has been attributed to a series of genetic bottlenecks in the domestication of chickpea, including the restricted distribution of the wild progenitor, the founder effect associated with domestication, and the shift from winter to summer cropping (Abbo et al., 2003). To compound this problem, genetic erosion of *C. arietinum* intraspecific resources is occurring due to the loss of local ecotypes, through diseases, insects, and environmental stresses, as well as for economic and strategic reasons (Malhotra et al., 2000; Abbo et al., 2003). Breeders are therefore looking to the wild relatives of chickpea as an alternative genetic resource for crop improvement.

The world collection of annual wild *Cicer* species is very limited and currently consists of 593 entries held in nine genebanks around the world. These represent only 285 known separate accessions of the eight annual wild *Cicer* species (Berger et al., 2003). Of these, only 120 have been collected independently from wild populations, with the remainder being selections from the original material. There is a single original accession of *C. cuneatum*, 2 of *C. chorassanicum*, 13 of *C. echinospermum*, 18 of *C. reticulatum*, 21 of *C. bijugum*, 28 of *C. pinnatifidum*, three of *C. yamashitae*, and 34 of *C. judaicum*. Thus, only a small proportion of the habitat and genetic diversity that is potentially available in wild populations is present in *ex situ* collections. It is essential to widen genetic diversity of the world collection of annual wild *Cicer* species by conducting targeted missions, which address the multitude of gaps in the current collection (Berger et al., 2003).

The world germplasm collection of perennial *Cicer* species is even more limited. Compared to the annual species, screening has been difficult due to the inherent problems with growing these species so far from their adapted climatic conditions. Malhotra et al.

(2000) have suggested that they should be conserved *in situ* by preserving their original habitats. Recent progress in determining adequate growing conditions for some of the perennial species, and subsequent establishment of perennial *Cicer* nurseries in eastern Washington state should go some way to improving this situation in the future (Kaiser et al., 1997). Approximately 12 of the 34 perennial species have been maintained, with various degrees of success, at The Germplasm Resources Information Network-United States Department of Agriculture (Pullman, WA).

7.4 TRAITS OF ECONOMIC SIGNIFICANCE WITHIN THE WILD *CICER* GENE POOL

Comprehensive screening of wild *Cicer* collections at the International Centre for Agricultural Research in Dry Areas (ICARDA), Syria, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India, and other national institutions have identified germplasm with resistance to various diseases and insects. Table 7.2 lists the traits of economic significance currently identified within the wild annual and perennial species of *Cicer*.

Wild *Cicer* species are the only source of resistance so far found for bruchid (*Callosobruchus chinesis* L.) and cyst nematode. They have a higher level of resistance than the cultivated species for fusarium wilt, leaf miner, phytophthora root rot, drought, ascochyta blight, pea streak carlavirus, and cold. Perhaps even more importantly, several accessions of the wild *Cicer* species are resistant to three or more stresses (Robertson et al., 1996). Particularly promising are the following accessions that are resistant to four or five different stresses, viz. *C. reticulatum* (ILWC 81, 112), *C. echinospermum* (ILWC 39, 181), *C. bijugum* (ILWC 32, 62, 73, 79), *C. judaicum* (ILWC 46), and *C. pinnatifidum* (ILWC 236). Such accessions are obvious targets for interspecific hybridization efforts. Overall, the species containing accessions with the highest levels of resistance to the most stresses in order of performance are: *C. bijugum, C. pinnatifidum,* and *C. judaicum* (Singh et al., 1994; Singh et al., 1998).

Accessions of the eight wild annual *Cicer* species have also been evaluated for various morphological traits at ICARDA and a catalogue was prepared (Robertson et al., 1995). Robertson et al. (1997) have reported useful variations for vegetative and reproductive characters in the annual wild species. Not surprisingly, *C. arietinum* showed greater intraspecific morphological variability compared with the wild species, particularly for characters like leaf area, growth habit, plant height, first pod height, pod dehiscence, and 100-seed weight. Among the wild species, *C. reticulatum* showed the largest overall morphological variability, followed by *C. pinnatifidum, C. echinosper-*

Table 7.2 Desirable Traits Identified in Wild Annual and Perennial *Cicer* Species

Trait	Species with Resistance/Tolerance	Reference(s)
Ascochyta blight	*C. anatolicum, C. bijugum, C. cuneatum, C. echinospermum, C. judaicum, C. montbretti, C. pinnatifidum*, C. reticulatum	Haware et al., 1992; Singh and Reddy, 1993; Stamigna et al., 1998; Singh et al., 1998; Collard et al., 2001; Collard et al., 2003a
Botrytis grey mould	*C. bijugum*	Haware et al., 1992
Bruchid	*C. bijugum, C. cuneatum, C. echinospermum,*	Singh et al., 1994; Singh et al., 1998
	C. judaicum, C. pinnatifidum, C. reticulatum	
Cold	*C. bijugum, C. echinospermum, C. judaicum, C. microphyllum, C. pinnatifidum, C. reticulatum*	van der Maesen and Pundir, 1984; Singh et al., 1990,1995; Singh et al., 1998; Toker, 2004
Cyst nematode	*C. bijugum, C. pinnatifidum, C. reticulatum*	Singh et al., 1989; Singh and Reddy, 1991; Singh et al., 1994, 1996; Singh et al., 1998; Di Vito et al., 1996
Crenate broomrape	*C. bijugum, C. canariensis, C. echinospermum, C. judaicum, C. macracanthum, C. multijugum, C. oxyodon, C. pinnatifidum, C. reticulatum, C. songaricum, C. yamashitae*	Rubiales et al., 2004
		C. bijugum, C. echinospermum, C. judaicum, C. pinnatifidum, C. reticulatum C. bijugum, C. canariense, C. cuneatum,
Drought	*C. microphyllum*	Chandel, 1984 .... implied based on distribution
Fusarium wilt	*C. bijugum, C. echinospermum, C. judaicum, C. pinnatifidum, C. reticulatum*	Nene and Haware, 1980; Haware et al., 1992; Singh et al., 1994; Kaiser et al., 1994; Infanito et al., 1996; Porta-Puglia and Infantino, 1997
Helocoverpa pod borer	*C. bijugum, C. canariense, C. cuneatum, C. echinospermum, C. judaicum, C. macracanthum, C. microphyllum, C.*	Kaur et al., 1999, Sharma et al., 2002, 2004

	pinnatifidum, C. reticulatum	
Leaf miner	*C. bijugum, C. chorassanicum, C. cuneatum, C. echinospermum, C. judaicum, C. pinnatifidum, C. reticulatum*	Singh and Weigand, 1994; Singh et al., 1994, 1998
Pea streak carlavirus	*C. anatolicum, C. canariensis, C. microphyllum, C. oxyodon*	Kaiser et al., 1993
Phytophthora root rot	*C. echinospermum*	Singh et al., 1994; Knights et al., 2003
Trypsin inhibitor	*C. chorassanicum*	Ahmad and Kollipara, 2004

mum, and *C. bijugum*. Of interest was the variability for wide leaflets in *C. chorassanicum*, number of branches in *C. bijugum*, and *C. reticulatum* and early flowering in *C. judaicum*.

In terms of nutritional value, there is a wide variation in seed protein and amino acid content in the annual *Cicer* species (Singh and Pundir, 1991). Seed protein ranges from 168 g/kg in *C. cuneatum* to 268 g/kg in *C. pinnatifidum*. Despite this, prospects for upgrading the nutritional value of chickpea by introgression of genes for high protein content are low, as the variability in the wild accessions falls within the range for the cultivated chickpea (Ocampo et al., 1998). Further, chickpea seed extract is known to inhibit trypsin and chymotrypsin (Saini et al., 1992), however, preliminary data for trypsin protease inhibitor indicate that there is no such activity in *C. chorassanicum* (Ahmad and Kollipara, 2004).

7.5 SPECIES RELATIONSHIPS AND INTERSPECIFIC HYBRIDIZATION

7.5.1 Species Relationships within the Genus

Traditionally phenotypic traits (Nozzolillo, 1985; De Leonardis et al., 1996; Robertson et al., 1997; Hassan, 2000; Javedi and Yamaguchi, 2004a), hybridization success (Ladizinsky and Adler, 1976a, 1976b; Pundir and van der Maesen, 1983; Verma et al., 1990; Pundir et al., 1992; Sheila et al., 1992; Pundir and Mengesha, 1995; Badami et al., 1997; Singh et al., 1999a, 1999b; Stamigna et al., 2000), analysis of chromosome pairing in hybrids (Ladizinsky and Adler, 1976a, 1976b; Ahmad et al., 1987; Ahmad, 1988), and the study of chromosome structure (Ohri and Pal, 1991; Ocampo et al., 1992; Tayyar et al., 1994; Ahmad, 2000) have been widely used methods for analysis of genomic relationships and the construction of phylogenies among *Cicer* species. Over the past 15 years, electrophoretic data based on seed storage protein (Ladizinsky and Adler, 1975; Vairinhos and Murray, 1983; Ahmad and Slinkard, 1992) and isozymes (Kazan and

Muehlbauer, 1991; Ahmad et al., 1992; Labdi et al., 1996; Tayyar and Waines, 1996; Gargav and Gaur, 2001, Sudupak and Kence, 2004) have also been applied to systematic studies in *Cicer*. More recently, DNA-based techniques (Patil et al., 1995; Sharma et al., 1995; Ahmad, 1999; Choumane et al., 2000; Iruela et al., 2002; Sudupak et al., 2002; Rajesh et al., 2003; Javedi and Yamaguchi, 2004a, b; Nguyen et al., 2004; Sudupak, 2004; Sudupak et al., 2004) have provided many new approaches to compare aspects of genome relationships in ways not previously possible.

Among the annual *Cicer* species, there is a consensus that *C. reticulatum* and *C. echinospermum* are the wild species most closely related to the domesticated *C. arietinum. Cicer bijugum, C. pinnatifidum,* and *C. judaicum* show a closer relationship among themselves and appear to form a group that is next closest to the first group containing the cultivated species. The remainder of the annual species, viz. *C. chorassanicum, C. yamashitae,* and *C. cuneatum* share an even more distant relationship with the cultivated species. This relationship only generally holds true, as the five annual species that are not part of the group with the cultivated species appear to change positions for relatedness to each other and to *C. arietinum*. Perennial *Cicer* species have only recently been subjected to phylogenetic analysis (Tayyar and Waines, 1996; Gargav and Gaur, 2001; Iruela et al., 2002; Sudupak et al., 2002; Rajesh et al., 2003; Javedi and Yamaguchi, 2004a, b; Nguyen et al., 2004; Sudupak, 2004; Sudupak and Kence, 2004; Sudupak et al, 2004). Again, with some exceptions, the perennial species in general have shown a distant relationship and are far removed from the cultivated species. The relationship between the perennial species *C. anatolicum* and domesticated chickpea remains a contentious issue, with some studies placing this species as a close relative of the cultivated species, while others indicating it to be far removed (Kazan and Muehlbauer, 1991; Staginnus et al., 1999; Choumane et al., 2000; Rajesh et al., 2003; Nguyen et al., 2004; Sudupak, 2004; Sudupak and Kence, 2004).

7.5.2 Gene Pools and Interspecific Hybridization

Ladizinsky and Adler (1976a, 1976b) and van der Maesen (1987) adopted the classical definition of the primary, secondary, and tertiary gene pools as proposed by Harlan and de Wet (1971) for classification of the *Cicer* wild relatives. According to this definition, the primary gene pool includes all species that hybridize freely, show good chromosome pairing leading to gene exchange, and produce viable hybrids. The secondary gene pool includes species that can be used as germplasm resources; however, hybridization with the cultivated species is difficult because of genetic barriers or chromosome alterations, and some degree of sterility is associated with the first-generation hybrids. Members of the tertiary gene pool are difficult to utilize, and sterility is always associated with hybrids. Fertility can sometimes be restored, but usually the percentage of recovered viable zygotes is extremely small.

On the basis of Harlan and de Wet's (1971) definition, and in consideration of the results obtained from crossability, biochemical and molecular diversity, and karyotypic studies, a recently revised model of the wild annual *Cicer* gene pools has been proposed (Croser et al., 2003a). However, if one were to follow Harlan and de Wet's (1971) definition alone, the primary gene pool of *Cicer* would consist of *C. arietinum* and only one wild species, the wild annual progenitor *C. reticulatum*. Although the other closely

related species, *C. echinospermum*, crosses quite readily with the cultivated species, it shows varying levels of sterility in the F_1 and F_2 generations. The secondary gene pool, thus, consists of *C. echinospermum* only. *Cicer bijugum*, *C. pinnatifidum*, and *C. judaicum*, which have been reported to give hybrids when crossed with the cultivated species (Verma et al., 1990; Singh et al., 1994; Singh et al., 1999a, 1999b), have been placed in the secondary gene pool by Croser et al. (2003b). This, however, is an issue that is not resolved yet and calls for some discussion. Although successful crosses of *C. bijugum*, *C. pinnatifidum*, and *C. judaicum* with *C. arietinum* have been reported, the authors have as yet failed to produce (except the *C. arietinum*×*C. judaicum* hybrid) (Verma et al., 1995) conclusive proof of hybridity by any cytogenetical, biochemical, or DNA-based approach. Indeed, there is a growing skepticism in the scientific community about the authenticity of these apparent partially fertile hybrids and their resulting progenies (Pundir, personal communication, 1994; Cubero and Ocampo, personal communication, 2004; Muehlbauer, personal communication, 2004). Considering the ongoing scientific dilemma and the lack of success, despite repeated attempts by various researchers globally utilizing embryo and ovule culture procedures and spanning more than two decades, we conclude that partially fertile interspecific hybrids and their progenies as claimed by the above research groups (Verma et al., 1990; Singh et al, 1994; Singh et al., 1999a, 1999b) are highly questionable. We would, thus, propose that the above three species should be placed in the tertiary gene pool of chickpea, along with the remaining annual species *C. chorassanicum*, *C. yamashitae*, and *C. cuneatum*. The lack of availability of perennial *Cicer* species has greatly restricted the assessment of their crossability with the cultivated species. Given the current situation with perennial *Cicer* species (Croser et al., 2003a), and until proven otherwise, we feel these species should be appropriately placed in the tertiary gene pool along with the six other annual wild species.

It is obvious from crossability studies of the cultivated species with the wild *Cicer* species that technical difficulties in obtaining hybrids beyond those within the primary and secondary gene pools remain a major obstacle. Apart from a few rather surprising exceptions (Verma et al., 1990; Singh et al., 1994; Singh et al., 1999a, 1999b), postfertilization incompatibility barriers (Bassiri et al., 1987; Ahmad et al., 1988; Stamigna et al., 2000; Ahmad and Slinkard, 2004) have restricted successful hybridization (using conventional crossing techniques) exclusively to the species of the primary and secondary gene pools. Given the fact that the tertiary gene pool species remain crossincompatible with the cultivated species, bridging crosses deserve further attention in chickpea in light of the known close relationships of *C. arietinum* with the wild annual species *C. reticulatum* and *C. echinospermum*. Ahmad and Slinkard (2003) have recently shown that, provided reciprocal crosses are used, there are at least no prefertilization barrier(s) limiting the hybridization of *C. reticulatum* and *C. echinospermum* with the other annual wild *Cicer* species. If the above-mentioned three species can be crossed with any one of the wild annual or perennial species, then it would be possible to transfer traits of interest to *C. arietinum*.

Rescuing abortive interspecific embryos and their *in vitro* culture has been proposed as an effective strategy for overcoming species barriers (van Rheenen, 1991; Muehlbauer et al., 1994; Singh et al. 1994; Robertson et al., 1996; Singh and Ocampo, 1997). In *Cicer*, there has been limited success from attempts to develop tissue culture techniques

to enable hybridization between chickpea and the more distantly related annual wild species *C. cuneatum, C. pinnatifidum,* and *C. bijugum* (Singh and Singh, 1989; Swamy and Khanna, 1991; Verma et al., 1995; Badami et al., 1997; van Dorrestein et al., 1998; Mallikarjuna, 1999, 2001). To date, a technique that enables the reproducible rescue and growth of embryos before eight days after pollination is lacking in *Cicer*. Thus, there is a clear need to identify and fine-tune information regarding the physiological, nutritional, and hormonal requirements of developing chickpea embryos (Croser, 2002; Croser et al., 2003a). An international collaboration has been established between Canadian, Australian, and Indian researchers to develop techniques for efficient interspecific hybridization between the cultivated and wild *Cicer* species.

7.6 CYTOGENETICS AND LINKAGE MAPS OF CHICKPEA

7.6.1 Cytogenetics

Chickpea is a crop that is not amenable to cytogenetic studies. Hence, most studies are limited to chromosome counts and feulgen-stained studies of karyotypes because of the small size and sticky nature of chromosomes. No cytogenetic stocks, other than tetraploids, are available in chickpea (Bahl, 1987; Gupta and Sharma, 1991) and except for one (Vlacilova et al., 2002), linkage groups have not yet been associated with respective chromosomes. *Cicer arietinum* has been the subject of a considerable number of karyotypic studies, while the wild annual species have received less attention (Croser et al., 2003a).

The nine annual and nine of the remainder 34 species are confirmed diploids with $2n=2x=16$ chromosomes (Table 7.1) (Ladizinsky and Adler, 1976a, 1976b; Ahmad, 1989; Pundir et al., 1993; Ohri, 1999; Ahmad, 2000; Ahmad and Chen, 2000). The karyotypes of the primary gene pool species *C. arietinum, C. reticulatum, C. echinospermum,* and the tertiary gene pool species *C. songaricum* and *C. anatolicum* show a high degree of similarity to each other and form the first group, while the second group contains all of the remaining six annual species (Ahmad, 2000; Croser et al., 2003a). Karyotypic details of the other species in the genus are not known. The crossability of these two perennial species with *C. arietinum* remains largely unknown. Whether a karyotypic similarity-crossability equation holds true in the *Cicer* genus needs additional research, but broad karyotypic and crossability studies of the cultivated chickpea with perennial *Cicer* species for alien gene introgression appear warranted.

Our understanding of the cytogenetic map in chickpea and related *Cicer* species is very much lacking. Individual chromosomes of chickpea are reported to be identifiable by C-banding and fluorochrome staining (Galasso and Pignone, 1992; Tayyar et al., 1994; Galasso et al., 1996). The related annual wild species have also been subjected to a solitary C-banding analysis (Tayyar et al., 1994), and the general conclusion drawn is that the heterochromatic C-bands are located proximally around the centromere with only occasional bands in intercalary and distal positions. Pachytene chromosome analysis of the cultivated species (Ahmad and Hymowitz, 1993) has further corroborated such distribution of heterochromatin. While the authors (Tayyar et al., 1994) were able to

identify individual pairs of chromosomes within a species, its applicability in an interspecific hybrid situation remains to be explored.

Karyotype analyses have shown that only one chromosome pair is associated with the nucleolar organizing regions, and is thus considered satellited (Ocampo et al., 1992; Ahmad, 2000). The satellite is present on the longest first chromosome in *C. arietinum*, *C. reticulatum*, and *C. echinospermum*, and an extra satellite in the case of *C. reticulatum* may also be present on the second chromosome. Unlike the somatic karyotype, detailed pachytene chromosome analysis had shown the third chromosome to be clearly satellited in *C. arietinum* (Ahmad and Hymowitz, 1993). Such refined pachytene analysis has made individual chromosome identification more conclusive and adds a further dimension to chickpea cytology (Ahmad and Hymowitz, 1993).

Given the poor state of chickpea cytology, there has been commendable progress in molecular cytogenetics in the genus. Individual chickpea chromosomes have been successfully sorted by flow cytometry (Vlacilova et al., 2002) and utilized for mapping specific DNA sequences and genes to individual chromosomes. Thus, specific genes (coding for various rRNA loci), major random repetitive DNA sequences, STMS markers, microsatellites, En/Spm-like transposon sequences, simple sequence repeats, and *Arabidopsis-type* telomeric sequences have been successfully hybridized to and localized on the chickpea chromosomes by fluorescent *in situ* hybridization (FISH) (Abbo et al., 1994; Galasso et al., 1996; Gortner et al., 1998; Staginnus et al., 1999, 2001; Vlacilova et al., 2002; Valarik et al., 2004). Using polymerase chain reaction and FISH, Vlacilova et al. (2002) have successfully associated two STMS markers (belonging to linkage group 8 of Winter et al., 2000) to the shortest chromosome of the chickpea genome. More recently, FISH analysis on super-stretched flow-sorted chickpea chromosomes has revealed spatial resolution of neighboring loci that has not been obtained by any other method (Valarik et al., 2004). It is expected that further technical advances will lead to the development in genome mapping of chickpea and the association of all genetic linkage groups to specific well-defined chromosomes.

7.6.2 Genetic Linkage Mapping

Development of high-density integrated genetic linkage maps based on morphological, biochemical, and DNA markers is a prerequisite for use in marker-assisted selection, positional cloning, and mapping of quantitative trait loci of agronomically important traits in a crop species. Although an impressive amount of progress has been achieved in linkage mapping, chickpea researchers are still awaiting a sufficiently dense genetic linkage map. Intraspecies polymorphism in cultivated chickpea for commonly used molecular markers is extremely low, therefore, interspecific crosses (*C. arietinum*×*C. reticulatum*, *C. arietinum*×*C. echinospermum*) have been exploited to develop genetic linkage maps with a higher number of markers (Gaur and Slinkard, 1990a, 1990b; Simon and Muehlbaur, 1997; Winter et al., 2000; Tekeoglu et al., 2002; Pfaff and Kahl, 2003). A genetic map constructed from an interspecific cross may not represent the true recombination distance map order of the cultivated genome due to uneven recombination of homoeologous chromosomes and distorted genetic segregation ratios (Flandez-Galvez et al., 2003a). A genetic linkage map constructed from a cross within the cultivated gene pool (Cho et al., 2002; Flandez-Galvez et al., 2003a), especially in the framework of

targeting traits of breeding importance, would therefore be more desirable. The genetic linkage maps developed to date with morphological, biochemical, and DNA-based molecular markers are summarized in Table 7.3.

The beginnings of linkage map development in chickpea were based on morphological and isozyme loci. However, their small numbers and the fact that expression of these markers is often influenced by the environment, makes them unsuitable for routine use. Thus, these maps were sparse and represented less than 30 loci mapped in a very small portion (about 250 cM) of the chickpea genome (Gaur and Slinkard, 1990a, 1990b; Kazan et al., 1993). Later developments on genetic linkage maps in chickpea that started with the work of Simon and Muehlbauer (1997) relied heavily on DNA-based molecular markers. In the lack of more recently available molecular markers, Simon and Muehlbauer (1997) employed RFLP and RAPD markers that show limited polymorphism in the cultivated species (Udupa et al., 1993; Banerjee et al., 1999).

Two independent interspecific-derived populations have been extensively employed for genetic linkage map development in chickpea (*C. arietinum* ICC 4958×*C. reticulatum* PI 489777 at the University of Frankfurt, Germany, and *C. arietinum* FLIP 84–92C×*C. reticulatum* PI 599072 at Washington State University, Pullman, WA), with the one developed in Germany being denser than the other. The 982 cM distance linkage map developed by Santra et al. (2000), which consisted of 89 RAPD, 17 ISSR, 9 isozyme, and 1 morphological markers was further expanded (Rajesh et al., 2002b; Tekeoglu et al., 2002) by integrating sequence tagged microsatellite sites (STMS) and resistance gene analog (RGA) loci. It covers a distance of 1175 cM. The STMS markers have proved very useful in linkage mapping and formed the basis for the map initially developed by Winter et al. (1999) that spanned a distance of 613 cM and consisted of 120 STMS markers. This map was greatly extended by Winter et al. (2000) and more recently by Pfaff and Kahl (2003) with the addition of 47 defense response (DR) genes. It covers a distance of 2500 cM arranged in 12 linkage groups and represents the most extensive linkage map in chickpea (Table 7.3). Relatively smaller maps derived from intraspecific (within *C. arietinum*) crosses, are also being developed (Cho et al., 2002; Flandez-Galvez et al., 2003a).

Combining these maps into a consensus linkage map is in progress and is being facilitated by the International Chickpea Genomics Consortium (Rajesh et al., 2004; http://www.icgc.wsu.edu/). Although steady progress is being made in extending the genetic linkage map in chickpea, there has been little effort in relating these maps to the eight pairs of chromosomes in the cultivated chickpea. Using flow-sorted chromosomes and molecular cytogenetic analysis, a recent report (Vlacilova et al., 2002) has linked STMS markers GAA46 and TS45 (linkage group 8 of the linkage map developed by Winter et al., 2000) to chromosome number 8 of chickpea.

Table 7.3 Genetic Resources Utilized in the Evolution of Genetic Linkage Map Development in Chickpea*

Publication	Mapping Population	Remarks (map size, markers)
Gaur and Slinkard, 1990a,	F_2, intraspecific *(C. reticulatum)*	200 cM, 7 linkage groups
1990b	F_2, interspecific *(C. arietinum×C. reticulatum)* and F2, interspecific *(C. arietinum×C. echinospermum)*	3 morphological and 26 isozymes
Kazan et al., 1993	F_2, intraspecific *(C. arietinum)*	257 cM, 8 linkage groups
	F_2, interspecific *(C. arietinum×C. reticulatum)* and F2, interspecific *(C. arietinum×C. echinospermum)*	5 morphological and 23 isozymes
Simon and Muehlbauer, 1997	F_2, interspecific *(C. arietinum×C. reticulatum)* and F2, interspecific *(C. arietinum×C. echinospermum)*	550 cM, 10 linkage groups
		9 morphological, 27 isozyme, 10
		RFLP and 45 RAPD
Winter et al., 1999	RIL, interspecific *(C. arietinum×C. reticulatum)*	613 cM, 11 linkage groups 120 STMS
Winter et al., 2000	Same as Winter et al.,1999	2078 cM, 16 linkage groups 118 STMS, 96 DAF, 70 AFLP, 37 ISSR, 17 RAPD, 8 isozyme, 3 cDNA, 2 SCAR and 3 morphological
Santra et al., 2000	RIL, interspecific *(C. arietinum×C. reticulatum)*	982 cM, 9 linkage groups 89 RAPD, 17 ISSR, 9 isozyme, and 1 morphological
Hajj-Moussa et al., 2001	RIL, interspecific *(C. arietinum×C. reticulatum)*	23 linkage groups RAPD, ISSR, and morphological
Rajesh et al., 2002b	Same as Santra et al., 2000	Addition of RGA Potkin 1–2 171 to Linkage group 5 of Santra et al. (2000)
Tekeoglu et al., 2002	Same as Santra et al., 2000	Extended map of Santra et al. (2000) to 1,175 cM

		50 STMS and 1 RGA was integrated
Cho et al., 2002	RIL, intraspecific *(C. arietinum)*	297 cM, 14 linkage groups 68 STMS, 34 RAPD, 4 ISSR, and 5 morphological
Pfaff and Kahl, 2003	Same as Winter et al. 1999; 2000	Incorporated 47 DR gene specific markers to Winter et al. (2000)
		2500 cM, 12 linkage groups
Flandez-Galvez et al., 2003a	F_2, intraspecific *(C. arietinum)*	535 cM, 8 linkage groups 51 STMS, 3 ISSR, 12 RGA
Collard et al., 2003b	F_2, interspecific *(C. arietinum×C. echinospermum)*	570 cM, 8 linkage groups 14 STMS, 54 RAPD, 9 ISSR, 6 RGA

* RIL: Recombinant Inbred Line, RFLP: Restriction Fragment Length Polymorphism, RAPD: Random Amplified Polymorphic DNA, STMS: Sequence Tagged Microsatellite Site, DAF: DNA Amplification Fingerprint, AFLP: Amplified Fragment Length Polymorphism, SCAR: Sequence Characterized Amplified Region, ISSR: Inter Simple Sequence Repeat, RGA: Resistance Gene Analog, DR: Defense Response.

7.7 GERMPLASM ENHANCEMENT

7.7.1 International Efforts in Chickpea Research

Systematic international efforts on chickpea research started after the establishment of two future harvest Centers of Consultative Group on International Agricultural Research (CGIAR). The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) was established in 1972, with its headquarters in Patancheru, India, and the International Center for Agricultural Research in the Dry Areas (ICARDA) was established in 1977, with its headquarters in Aleppo, Syria. ICRISAT works on both *desi* and *kabuli* chickpeas, while ICARDA concentrates on *kabuli* chickpea. In collaboration with National Agricultural Research Systems (NARS), these centers have made extensive efforts on collection, characterization, and conservation of chickpea germplasm. The genebank of ICRISAT has more than 17,000 accessions of the cultivated species and 136 accessions of 18 wild species; similarly the genebank of ICARDA has more than 12,000 accessions of the cultivated species and 260 accessions of 8 wild species. These precious assets held, in trust for humanity, by these centers are provided freely to research and development specialists around the world. More than 116,000 seed samples of germplasm accessions from ICRISAT and more than 19,000 from ICARDA have been disseminated to requesting NARS. Given the large number of accessions available in the genetic resource collection, it becomes a daunting task to identify germplasm that could be used in a crop improvement program. A chickpea core collection, which is a chosen subset of large germplasm collection that generally contains about 10% of the total accessions and represents the genetic variability of the entire germplasm collection, as

recently developed by Upadhyaya and Ortiz (2001) and Upadhyaya et al. (2001), should greatly facilitate global chickpea breeding and genetic improvement.

Fortunately, in chickpea, 15 genebank accessions supplied by ICRISAT and 17 supplied by ICARDA have been directly released as varieties by national authorities in different countries. Additionally, ICRISAT and ICARDA have been helping the chickpea breeding programs of NARS around the world by making available segregating materials and advanced breeding lines. A total of 50 varieties released in 10 countries are from ICRISAT-supplied breeding materials. Similarly, 97 varieties in 25 countries are from ICARDA-supplied breeding material.

7.7.2 Conventional Breeding Efforts

7.7.2.1 Breeding Methods

The breeding methods used in chickpea are not different from other self-pollinated food legumes. In the early phase of chickpea breeding, most varieties were developed through selection from the landraces collected in the country or through germplasm introduction, evaluation, and selection. Later, the emphasis gradually shifted to hybridization for increasing genetic variation in the breeding materials. Most recent varieties have been developed through hybridization.

Single, three-way, double, or multiple crosses are used, depending on the number of traits to be combined and their spread in the parents. It is well established that selection for yield in early segregating generations is not effective in chickpea because of its indeterminate growth habit. Thus, many scientists prefer to select crosses rather than plants within crosses in early segregating generations (F_2 and F_3). The pedigree method, earlier used at many institutes, including ICRISAT, is not practiced widely in its original form, because it is cumbersome and only a limited number of crosses can be handled by this method. The bulk method, variously modified, is now the most common selection method used after hybridization in chickpea. Single-seed descent (SSD) method, a modification of bulk method, is widely used for development of recombinant inbred lines (RILs) for genome mapping studies. Backcross method is commonly used to incorporate one or few traits from a germplasm line, sometimes a wild species, to a well-adopted variety. A population improvement method that involves intercrossing of selected plants in F_2s or F_3s has been suggested for legumes for enhancing the chances of recombination in segregating generations (Muehlbauer et al., 1988). van Rheenen et al. (1991) proposed a method called polygon breeding, whereby segregating populations and selections are shared and exchanged between breeders. This method has been used by ICRISAT in collaboration with some state agricultural universities in India.

Attempts have been made to define chickpea ideotypes for different growing conditions. In the drought-prone environments, traits that help plants to escape or tolerate drought should be considered in the ideotype. Saxena and Johansen (1990) suggested that an ideotype for drought-stress environments should have early maturity, a deep root system, and a smaller leaf size. In the Mediterranean climate, the crop experiences a cool and wet winter followed by rapid warming in spring, leading to terminal drought. Thus, the ideotype for the Mediterranean environments should

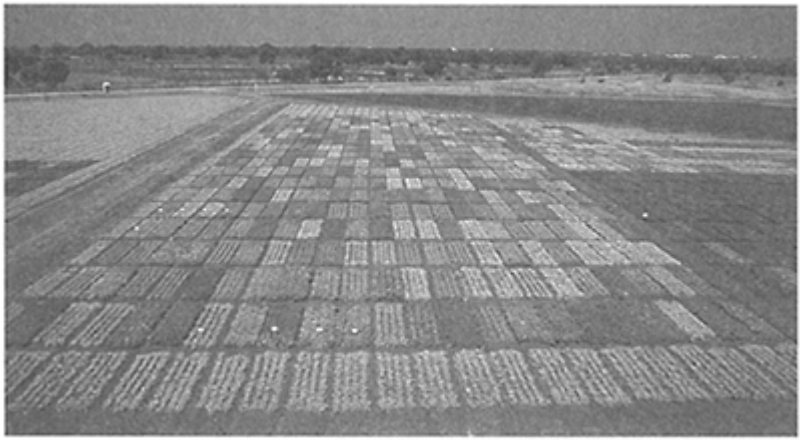

Figure 7.1 (See color insert following page 178) Breeding chickpea for early maturity at ICRISAT. Short-duration varieties have helped adaptation of chickpea in tropical environments.

include early flowering and tolerance to cold during flowering (Sedgley et al., 1990). It has also been suggested that a compact plant type with erect growth habit and short internodes could help resist excessive growth in high input conditions (Dahiya and Lather, 1990). A spontaneous mutant with short internodes and compact growth habit, E100YM, has been identified (Dahiya et al., 1984) and used in ideotype breeding. Promising progenies with compact growth habit have been obtained, which can be grown at high plant density (Lather, 2000).

7.7.2.2 Breeding for Tolerance to Abiotic Stresses and Widening of Adaptation

Drought is the most important constraint to yield in chickpea accounting for 40 to 50% yield reduction globally. Four approaches are being pursued: (1) high root mass, (2) smaller leaf area, (3) osmoticum adjustment, and (4) early-maturing short-duration varieties. Lines with a greater degree of drought tolerance have been developed by combining large root traits of ICC 4958 with fewer pinnules trait of ICC 5680 (Saxena, 2003). A recent screening of the mini-core collection has identified several other lines with large root traits (Krishnamurthy et al., 2003). Genotypic variation for osmotic adjustment has been observed in chickpea (Morgan et al., 1991; Leport et al., 1999; Abbo et al., 2002b). The heritability of osmotic adjustment has been found to be low (h^2 =0.20 to 0.33), indicating that gains from selection for increased osmotic adjustment are likely to be small (Abbo et al., 2002b). ICRISAT has placed a high emphasis on development of short-duration varieties (Figure 7.1), as these can escape terminal drought. The first

breakthrough was the development of an extra-short-duration *kabuli* variety, ICCV 2, from a multiple cross involving five parents (2 *kabuli* and 3 *desi*), which has helped the expansion of *kabuli* chickpea cultivation to tropical environments. Now ICRISAT has developed super-early chickpea lines (ICCV 96029, 96030) in *desi* chickpea, and these are being used extensively in crossing programs by NARS in India and Canada for development of early varieties.

Singh (1990) listed several advantages of winter chickpea in comparison to spring chickpea, including higher germination rate, less incidence of fusarium wilt, increased nitrogen fixation, better utilization of available moisture, mechanization of harvesting due to increased crop height, and increased protein and grain yield per hectare. The effects of low-temperature stress and its implications for chickpea improvement were reviewed recently by Croser et al. (2003b). Most chickpea cultivars are susceptible to chilling temperature at flowering (Croser et al., 2003b). The germplasm line ILC 8262, the mutant ILC 8617, and the breeding line FLIP87–82C were the best sources of cold tolerance in cultigen, with a consistent score of 3 (on a 1-to-9 scale) over years and locations (Singh et al., 1990, 1995). A number of varieties combining cold tolerance and resistance to ascochyta blight have been released in several countries from the breeding material supplied by ICARDA. A pollen selection method, as described by Clarke et al. (2004), has also been successfully used to transfer cold tolerance from ICCV 88516 (CTS 60543) to the popular varieties Amethyst and Tyson in Australia (Clarke and Siddique, 2004).

Legumes, in general, are sensitive to salinity, and within legumes, chickpea, faba bean, and field pea are more sensitive than other grain legumes. Saline soils are very common in West and Central Asia and Australia, where chickpea is widely grown. Soil salinity adversely affects germination, resulting in poor plant stand. Only salt tolerant cultivars can be grown successfully in soils having ECe higher than 4.0 dS/m. The levels of salinity tolerance identified in chickpea are low to moderate. Several tolerant sources have been identified in India and Pakistan (Singh and Singh, 1984; Dua and Sharma, 1995; Kathiria et al., 1997). The salt-tolerant lines CSG 88101and CSG 8927 identified by Dua and Sharma (1995) had lower Na^+ in root than the sensitive genotypes. A salinity tolerant *desi* chickpea variety Karnal Chana 1 (CSG 8963) has been released in India for salt-affected soils of northwestern parts.

7.7.2.3 Breeding for Resistance to Biotic Stresses

7.7.2.3.1 Diseases

Ascochyta blight (AB), caused by *Ascochyta rabiei* (Pass.) Labr. and represented by several pathotypes, is a highly devastating foliar disease of chickpea in West and Central Asia, North Africa, North America, and Australia. The problem of fungal variability and the existence of races appear to be complex. A standard set of well-characterized genotypes, a common inoculation technique, and a well-defined disease-rating methodology should be used by workers who wish to determine the extent and distribution of variability in *A. rabiei* in different geographic regions (Haware, 1998). Considerable efforts have been made on identification of chickpea genetic resources resistant to AB and breeding for AB resistance. Multilocation evaluation of chickpea

germplasm indicated that *kabuli* germplasm generally shows higher resistance to AB than *desi* germplasm (Reddy et al., 1992; Haware et al., 1995). Extensive germplasm screening has identified many resistant *desi* and *kabuli* lines (Reddy and Singh, 1984; Singh et al., 1984b). Using the breeding material supplied by ICARDA and ICRISAT, more than 100 AB tolerant varieties have been released in 25 countries. The national breeding programs in the U.S., Canada, Australia, Pakistan, India, Europe, and Western Asia have also made good progress in development of AB-resistant varieties.

Fusarium wilt (FW) is the most important root disease of chickpea. It has been reported from almost all the chickpea-growing regions of the world. Seven races of FW have been reported worldwide (Phillips, 1988; Jimenez-Diazet, et al., 1989). Effective field, greenhouse, and laboratory procedures for resistance screening have been developed (Nene et al., 1981) (Figure 7.2), and good progress has been made in identifying sources of resistance (Haware et al., 1990; van Rheenen et al., 1992). A number of varieties with absolute resistance to FW are available in many countries. In most cases, the resistance to FW has been stable. Some of the varieties released in India about two decades ago, such as JG 315 and JG 74, still maintain a high level of resistance.

Botrytis gray mold (BGM), caused by the necrotrophic fungus *Botrytis cinerea* Pres., is an important foliar disease of chickpea in northern India, Nepal, Bangladesh, and Pakistan (Haware and McDonald, 1992) and has been reported from more than 15 countries (Nene et al., 1996), including Australia (Corbin, 1975) and North America (Kharbanda and Bernier, 1979). High levels of resistance have not been found in the cultivated species (Haware and Nene, 1982; Haware and McDonald, 1993). Some accessions with erect plant type, such as ICCL 87322 and ICCV 88510, were found to be less affected by the disease (Haware and McDonald, 1993).

Figure 7.2 A Fusarium wilt screening nursery at ICRISAT.

Three types of root rot diseases (dry root rot, collar rot, and phytophthora root rot) are known to affect chickpea. High soil moisture, the presence of undecomposed organic matter on the soil surface, low soil pH, and a high temperature favor collar rot. There has been limited research toward the identification of races of dry root rot, even though the existence of different races has been implicated (Than et al., 1991). Pundir et al. (1988) provided a list of 47 tolerant accessions available in the genebank of ICRISAT. A few germplasm lines and cultivars have been identified to have low to moderate levels of resistance. These include the cultivars SAKI 9516 (Dua et al., 2001), breeding lines RSG 130, 132, and 191 (Chitale et al., 1990), and germplasm accessions ICC 1696, ICC 4709, and ICC 14391 (Singh, personal communication, 2003). The breeding efforts have led to release of a number of moderately resistant varieties, such as ICCC 37, ICCV 10, JG 130, and WCG 1 (Dua et al., 2001). Phytophthora root rot, caused by *Phytophthora medicaginis* Hansen, is the major disease of chickpea in northern New South Wales and Queensland (Knights et al., 2003). Genotypic differences in resistance to Phytophthora root rot have been identified (Brinsmead et al., 1985; Dale and Irwin, 1991), and cultivars less susceptible to the disease, such as Jimbour, have been developed. The levels of resistance available in the cultivated species are low as compared with the wild species (Knights et al., 2003).

7.7.2.3.2 Root Nematodes and Insect Pests

The major nematodes known to affect chickpea are root knot nematodes (*Meloidogyne* spp.), cyst-forming nematodes (*Heterodera* spp.), and lesion nematodes (*Prathylenchus* spp.). None of the 8000 accessions of cultivated chickpea (7000 *kabuli* type and 1000 *desi* type) screened at ICARDA against cyst nematodes was found to have even a moderate level of resistance (Di Vito et al., 1988). However, good sources of resistance have been identified in the wild species (Robertson et al., 1995).

Pod borer (*Helicoverpa armigera* Hubner) is the most important insect pest of chickpea worldwide. Several techniques are available for screening of chickpea genotypes for resistance to pod borer (Sharma et al., 2003). Only low to moderate levels of resistance for pod borer have so far been identified in the cultivated and wild *Cicer* species (Lateef and Pimbert, 1990; Sharma et al., 2002). Unavailability of a high level of resistance to pod borer in the cultivated and cross-compatible wild species has been the major limitation in developing pod borer resistant varieties. Limited progress has been made in breeding for resistance to leaf miner (Malhotra et al., 1996; Robertson et al., 1995). Seed beetle or bruchid (*Callosobruchus* spp.) is the most important storage pest of chickpea. No source of resistance to bruchid has been identified in the cultivated species. Some of the accessions of wild species showed no damage by this insect (Robertson et al., 1996). It is yet to be established whether the resistance is due to seed morphology or chemical properties.

7.7.2.4 *Introgression of Economically Valuable Traits through Wide Crosses*

Interspecific hybridization has played an important role in genetic enhancement of many crop species by facilitating transfer of useful traits to the cultivated species from wild,

weedy forms. However, the progress in chickpea is still in its infancy (Croser et al., 2003a). Although the wild annual *Cicer* species have been extensively evaluated, their exploitation in chickpea breeding programs has not yet been widely realized. No perennial species has yet been successfully hybridized with the cultivated species.

The exploitation of the wild *Cicer* species in breeding programs has been limited to cyst nematode (*Heterodera ciceri* Vovlas, Greco & Di Vito) resistance (Di Vito et al., 1996; Singh et al., 1996; Erskine et al., 2001; Malhotra et al., 2002). Selections derived from the interspecific hybrid of *C. arietinum*×*C. echinospermum* and possessing resistance to root lesion nematode, enhanced phytophthora resistance, and moderate resistance to ascochyta blight are also being considered for release in Australia (Knights et al., 2002).

In addition to the genes for resistance and tolerance, heterosis for yield has been observed in populations from crosses between *C. arietinum, C. reticulatum,* and *C. echinospermum* (Jaiswal et al., 1987; Singh and Ocampo, 1993, 1997). While, most introgressions have involved the two crosscompatible species, *C. reticulatum* or *C. echinospermum,* Singh et al. (1994), and Verma et al. (1995) are the only ones reporting F_2 recombinants with a very large number of secondary branches, high pod number, and yield from a cross between *C. arietinum* and *C. judaicum,* or *C. pinnatifidum,* or *C. bijugum*. Some progress has also been made in producing early maturing segregants, following interspecific hybridization. Transgressive segregants for days to flowering have been isolated from the interspecific cross *C. arietinum*×*C reticulatum* (Singh et al., 1984a).

An interesting perspective has been brought forth by Abbo et al. (2002a), who recently presented evidence indicating that some wild *Cicer* relatives have a vernalization requirement. This may be useful in certain environments to avoid early flowering and consequent frost damage, etc.; however, it also can be an unfavorable trait in other environments. Introgression of genes from the wild species back into chickpea may result in reintroduction of these vernalization-sensitive alleles into the cultivated species and should be considered with caution in any hybridization program.

7.7.2.5 Mutation Breeding

Most studies on polymorphism of molecular markers in chickpea indicate presence of limited genetic variability in the cultivated species, and this has forced researchers to use interspecific crosses for genome mapping (Gaur and Slinkard, 1990a, 1990b; Kazan et al., 1993; Simon and Muehlbauer, 1997; Winter et al., 1999, 2000). Kenneth J.Frey (Iowa State University, Ames, IA) called chickpea “a recalcitrant crop species,” as it has not been very amenable to genetic improvement in spite of extensive breeding efforts during the past three decades (van Rheenen et al., 1993). It was suggested that mutation breeding and interspecific hybridization should be used for increasing genetic variability and yield advancement of chickpea (van Rheenen et al., 1993).

A large variability is seen in chickpea germplasm for morphological traits, but it could be a reflection of the expression of a limited number of mutant genes, as a single mutant gene may cause marked changes in the appearance of the plant (Gaur and Gour, 2003). Though a variety of physical and chemical mutagens have been used, the most commonly used mutagen is γ-rays, with doses ranging from 10 to 40 kR. There are reports

suggesting that *kabuli* chickpeas are more sensitive to mutagens than the *desi* type (Khanna and Meharchandani, 1981; Kharkwal, 1998).

At least two institutes, the Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan and the Indian Agriculture Research Institute (IARI), New Delhi, India, have had strong programs on mutation breeding for incorporating disease resistance for chickpea improvement. The first of the three *desi-type* Ascochyta blight-resistant varieties, CM 72 from the NIAB program, was released in 1983, which was then followed by CM 88 in 1994, and CM 98 in 1998 (Haq et al., 1999). A *kabuli* variety, CM 2000, from this program was released in 2000. In India, at least six chickpea varieties have been developed through mutation breeding. Of these, three were developed by IARI. The mutation breeding program of IARI has had a major focus on resistance to diseases. The varieties, Pusa 408 (Ajay) and Pusa 413 (Atul), were moderately resistant to AB, whereas Pusa 417 (Girnar) was resistant to wilt and moderately resistant to stunt and root rots (Kharkwal et al., 1988; Micke, 1988; Dua et al., 2001). Three other chickpea mutants, RS 11, RSG 2 (Kiran), and WCG 2 (Surya), were developed by agricultural universities (Micke, 1988; Dua et al., 2001).

Mutation breeding has also helped to improve the nutritional quality of chickpea. The Bangladesh Institute of Nuclear Agriculture, Mymensingh, obtained a high-yielding and high-protein mutant of chickpea cultivar Faridpur-1 through γ-rays induced mutation. The mutant had 20% higher yield and 20% higher protein than the parental cultivar Faridpur-7. The mutant was released as a cultivar by the name “Hyprosola” in 1981 by the National Seed Board of Bangladesh (Oram et al., 1987). These examples clearly indicate that mutation breeding has played important role in chickpea improvement. In addition to the release of 11 mutants as cultivars, several mutants have been used as parents in breeding programs.

7.7.2.6 Development of Hybrids Using Cytoplasmic Male Sterility (CMS) System

Male sterility has been reported in chickpea (Chaudhary et al., 1970; Sethi, 1979). Genetic studies indicated that the male sterility was under the control of a monogenic recessive gene (Chaudhary et al., 1970; Reddy and Reddy, 1997). It has also been possible to induce male sterility (up to 80% pollen sterility) through gametocide (Mathur and Lal, 1999).

Utilization of male sterility for hybrid seed production requires an efficient mechanism for pollen dispersal by the male parent and reception of pollen by the stigma of the male sterile line. This is the biggest hurdle in chickpea, where cross-pollination has been reported to be less than 1% (Tayyar et al., 1995) due to the cleistogamous nature of the flower. Thus, the availability of CMS and fertility restorer systems will not be enough in chickpea for production of hybrid seed. A significant change in floral morphology is needed in the A, B, and R lines. It basically requires open flowers in which stigma and anthers are not enclosed by the petals. A male sterile open flower mutant has been reported in chickpea (Pundir and Reddy, 1988). It may be used as female parent. A similar, but male fertile, mutant is needed for use as male parent. A fertile outwardly curved-wing mutant has been reported (Gaur and Gour, 2003), but the keel petal still encloses the anthers in this mutant.

The commercial cultivation of hybrid chickpea may still be far from reality, even if we have the appropriate CMS system and the required changes in floral morphology. It will be difficult to produce a large quantity of hybrid seed as each pod produces only one or two seeds, and seed-to-seed multiplication is notoriously low in chickpea and other cool season grain legumes, compared to cereals and canola. Most chickpea cultivation (95%) is in developing countries where the majority of farmers cannot afford expensive hybrid seed every year. As there is no immediate hope of utilization of a male sterility system for development of commercial hybrids in chickpea, apomixis can be explored as an alternative system for exploitation of heterosis in this important food legume. So far, however, there is no report detailing the occurrence of apomixis in chickpea.

7.7.3 Genetic Transformation

The advancement in recombinant DNA technology and availability of efficient transformation and regeneration systems in plants have made it possible to transfer genes from any organism to plants with optimized expression. Targeted transfer of genes from the wild *Cicer* species into the cultivated species would represent a very elegant application of transformation technology. The progress in molecular mapping and, to some extent, chickpea transformation, now considered a routine procedure in chickpea, has brought the application of this type of technology much closer to reality in chickpea (Fontana et al., 1993; Hamblin et al., 1998; Chakrabarty et al., 2000; Krishnamurthy et al., 2000; Sharma and Ortiz, 2000; Jaiwal et al., 2001; Senthil et al., 2004). The applicability of this technology will, however, depend on the identification of key genes, the number of genes conferring a particular character, and public acceptance of cultivars resulting from transformation technology. In chickpea, transgenic technology is being exploited primarily for insectpests and disease resistance, drought tolerance, and quality enhancement.

An efficient and reproducible tissue culture regeneration system is a prerequisite for development of transgenics. Many earlier studies on chickpea tissue culture encountered problems in rooting or establishment of plants in the soil. One option tried was to graft the *in vitro* germinated shoot on scions of pre-germinated seedling (Krishnamurthy et al., 2000), although such a tedious method would be inefficient for routine use. Novel rooting systems that give a rooting frequency of 90% or more have been reported. These involve either placing elongated shoots on a filter paper bridge immersed in liquid rooting medium (Jayanand et al., 2003) or placing nodal segments in an inverse polarity in a specially formulated tissue culture medium (Fratini and Ruiz, 2003).

Several studies have been conducted on transformation of chickpea during the past decade. Reports are now available on successful transformation of chickpea and regeneration of transformed plants. The most commonly used method has been the *Agrobacterium-mediated* transformation using embryo axes as explants (Fontana et al., 1993; Kar et al., 1996; Chakrabarty et al., 2000; Krishnamurthy et al., 2000; Chandra and Pental, 2003). However, bombardment with accelerated tungsten particles has also been successfully used (Hussain et al., 1997; Kar et al., 1997; Tewari-Singh et al., 2004). Except Kar et al. (1997), all these studies used only marker genes like *uidA* and *npt II* for transformation. Sommers et al. (2003) are of the opinion that, in addition to the limited availability of an efficient transformation protocol, a rapid and reliable selection strategy

has also impeded the uptake of this technology in chickpea improvement programs. An efficient and reliable nonantibiotic selection strategy using the phosphinothricin-acetyltransferase and aspartate kinase genes, has been recently developed for the production of transgenic chickpea (Tewari-Singh et al., 2004).

One of the areas, where traditional chickpea breeding methods have yet to make progress is the development of cultivars resistant to the devastating pest helicoverpa pod borer. It is simply because sources of a high level of resistance are not available in the cultivated or cross-compatible wild species. Thus, development of transgenic chickpea seems to be the only hope to host-plant resistance to this insect-pest. There is a report (Kar et al., 1997) on development of transgenic chickpea plants for *BtCry1A(C)* gene derived from the bacterium *Bacillus thuringiensis* (Bt). The transformation method used was particle bombardment of embryo axis (devoid of root and shoot meristems) from mature seeds. The transformation was confirmed through molecular analysis. Insect feeding assay indicated inhibition of development of feeding larvae. There is no further report available on field testing of these transgenics.

ICRISAT has produced transgenics for resistance to pod borer by using *BtCry1Ab* and *SbTi* (soybean trypsin inhibitor) genes. The molecular characterization and insect bioassays are currently ongoing (Sharma, personal communication, 2003). New constructs with combinations of different *Cry* genes are being developed in collaboration with CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement) France, besides the artificial synthesis of some of these genes for optimal expressions in plants (Lavanya et al., 2003).

Research at the Scottish Crops Research Institute, U.K., has shown that immature raspberry fruit contains a polygalacturonase inhibitory protein (PGIP) effective against endopolygalactoseuronases produced constitutively by *Botrytis cinerea* (Johnston et al., 1993). Polygalactuseuronases are the key enzymes in the invasion of plant tissues by many facultative fungal pathogens. At ICRISAT, PGIP gene and other antifungal genes, such as chitinases and glucanases, are being introduced into chickpea for resistance to fungal diseases (Sharma and Ortiz, 2000). Efforts are also being made to identify and clone tissue-specific promoters for more controlled expression of these potential transgenes. A project funded under the Indo-Swiss Collaboration in Biotechnology (ISCB) is aimed at developing transgenic chickpea for tolerance to drought and low-temperature stresses by using genes with regulatory functions, such as drought responsive elements (DREs), and osmoregulation, such as *codA* and *P5CSF* (Sharma, personal communication, 2003). Research in progress in this area is expected to improve chickpea for the traits that are difficult to improve through traditional breeding methods. The applicability of this technology will, however, depend on the identification of key genes, the number of genes conferring a particular character, and public acceptance of cultivars resulting from transformation technology.

In addition, Sagare and Krishnamurthy (1991) have proposed fusion of protoplasts as an alternative means of enabling interspecific hybridization between widely related *Cicer* species. This technique is currently limited by the absence of protocols for the regeneration of whole plants from single cells or protoplasts in any of the *Cicer* species. Recent progress toward the development of a microspore culture system in *Cicer* (Croser et al., 1999; Lülsdorf et al., 2001; Croser, 2002; Croser and Lülsdorf, 2004) resulting in

embryogenesis from single-celled microspores in liquid culture may assist in the future development of this technique.

7.7.4 Molecular Marker-Assisted Selection

The underlying principle of molecular marker-assisted selection is that if a gene(s) is linked to an easily identifiable genetic marker, then it may be more efficient to select in a breeding program. This effectiveness, however, will depend on the strength of linkage of the marker to the locus controlling the character of interest. Such an approach can be the most effective means of enhancing the transfer of a desired gene(s) by a backcross program, reducing the linkage drag resulting from a wide cross, and for pyramiding genes and alleles of various combinations. The successful application of marker technologies, thus, requires (1) a dense saturated intraspecific map linking traits of agronomic importance to highly polymorphic, co-dominant markers in sufficiently close proximity to allow marker-assisted selection in offspring derived from intraspecific crosses, and (2) tailoring of markers and reactions in combination with high-thoroughput screening techniques to speed up and facilitate the application of marker technology.

The history of developing and identifying molecular markers for marker-assisted selection in chickpea, understandably reflects a mirror image of the increasing understanding of genetic linkage maps in the crop. The first of such markers was not identified until 1997 (Mayer et al., 1997), however, recent progress has resulted in many markers being identified (Table 7.4). Indeed, the developments in chickpea have lagged considerably behind those in cereals (Gupta et al., 1999). Other than the molecular markers identified for double podding trait (Cho et al., 2002; Rajesh et al., 2002a), leaf traits, and erect growth habit (Banerjee et al., 2001), only genes conferring resistance to fusarium wilt and ascochyta blight have been tagged. Most of the populations utilized in genetagging experiments have been derived from interspecific crosses with the wild annual species *C. reticulatum* and, to a limited extent, *C. echinospermum*, as well as some intraspecific crosses within the cultivated species. Among the various types of molecular markers that are available, the STMS markers are most amenable for multiplex PCR and electrophoresis, as shown for more than 600 STMS markers in soybean (Cregan et al., 1999; Narvel et al., 2000). In chickpea, the number of STMS markers is still too low for such applications. Marker development is in progress at the various research institutions working on chickpea, however, there is no application of a robust

Table 7.4 Potentially Useful Markers for Marker-Assisted Selection in Chickpea Improvement

Publication	Marker	Remarks
Mayer et al., 1997	RAPD marker UBC-170_{550}	7 cM from fusarium wilt resistance to race 1
Ratnaparkhe et al., 1998b	RAPD marker UBC-855_{500}	Linked in repulsion, 5.2 cM from fusarium wilt resistance to race 4
Ratnaparkhe, et al.,1998b	ISSR marker UBC-825_{1200}	5.0 cM from fusarium wilt resistance to race 4
Tullu et al., 1998	RAPD marker CS-27_{700}	9 cM from fusarium wilt resistance to race 1
Tullu et al., 1999	RAPD marker CS-27_{700}	Fusarium wilt resistance to race 4
Santra et al., 2000	2 RAPD marker	10.9 cM apart and flanking major QTL-1 for ascochyta blight resistance
	1 ISSR and 1 isozyme	5.9 cM apart and flanking major QTL-2 for ascochyta blight resistance
Banerjee et al., 2001	RAPD markers	Linked to QTL for leaf length, leaf width, and erect plant habit
Cho et al., 2002	PCR marker Tr44	7.8 cM from gene for double podding trait
	QTL marker Ta130s	Explaining 31% of the variation for seed number per plant
Rajesh et al., 2002a	STMS marker T-80	4.84 cM from gene for double podding trait
Benko-lseppon et al., 2003	SCAR marker R-2609–1	2.0 cM from fusarium wilt resistance to race 4
Collard et al., 2003b	QTL marker CS44a$_{1150}$	Significant QTL for seedling resistance to ascochyta blight
Flandez-Galvez et al., 2003b	6 QTL markers	For ascochyta blight resistance
Idnani and Gaur, 2003	Isozyme marker *Amy*	6.2 cM from leaf necrosis gene *(nec)*
Millan et al., 2003	19 RAPD, 1 ISSR markers	Distributed in an 18.8 cM region, and resistance to ascochyta blight,
	QTL marker	marker OPAC/1200 present most frequently
Rakshit et al., 2003	DAF marker OPS06–1	Significant QTL for ascochyta blight resistance

Rubio et al., 2003	RAPD marker $OPJ20_{600}$	6 cM from fusarium wilt resitance to race 0
Sharma et al., 2004	STMS markers TA96, TA27	0.6 cM from fusarium wilt resistance to race 3

molecular marker system for selection within a large breeding program. We anticipate that this technology is not far away, and it holds great potential for targeted introgression of genes from wild *Cicer* into chickpea.

7.8 SUMMARY

The rich diversity for various biotic and abiotic stresses present in the annual and perennial wild related species has to be exploited for genetic enhancement of chickpea. Wide hybridization is currently limited by reproductive barriers and simultaneous transfer of undesirable characters. The perennial species, in particular, are poorly evaluated and in many ways an unknown quantity. There is a clear need to develop methods for the maintenance and creation of *ex situ* collections of perennial species to enable their evaluation for important agronomic traits. An efficient embryo rescue protocol to enable crosses between chickpea and the more distantly related tertiary gene pool *Cicer* species will be crucial to the utilization of traits from these wild relatives. Recent advances in biotechnology techniques, including embryo rescue for interspecific hybrids, linkage mapping, marker-assisted breeding, and transformation, hold much promise for successful introgression of genes from wild annual species into cultivated chickpea in the future. The development and commercialization of genetically transformed chickpea, however, should be approached with caution, keeping in mind their possible rejection by consumers. There is little doubt that wild related *Cicer* species will play an important role in the future improvement of chickpea varieties. The task at hand is to identify the novel genes and find the best strategy to utilize them in a breeding program. The challenges facing the utilization of *Cicer* genetic resources are global and call for a wider collaborative research initiative for maximizing resource management and gains.

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CHAPTER 8
Lentil (*Lens culinaris* Medik.)

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8.1 INTRODUCTION

Lentil (*Lens culinaris* Medik.) is an old world grain legume food crop that was domesticated in the Near East arc along with other pulses such as pea, chickpea, and faba bean in early Neolithic times (Helbaek, 1959; Ladizinsky, 1979). Lentil co-evolved with wheat and barley, which became staple crops for Neolithic agriculture more than 7,000 years ago. Lentil is adapted to semi-arid environments, where it is usually grown in rotations that include cereals; however, regions of production encompass all continents except Antarctica. As a preferred food legume, lentil has numerous common names including *adas* (Arabic), *mercimek* (Turkey), *messer* (Ethiopia), *masser* (India), and *heramame* (Japanese), to name a few. Lentil has a protein concentration estimated at 24% and ranging from 22 to 26%; however, the protein is lacking in sulfur-containing amino acids but has relatively large amounts of lysine (Newman et al., 1988). For comprehensive reviews of nutritional quality and utilization of lentil, see Huisman et al. (1994) and Hulse (1994). Lentil is relatively fast cooking and is a nutritious food that is highly popular, especially in semi-arid regions of the Middle East, North Africa, and South Asia.

The crop is currently produced on 3.8 million hectares worldwide, with production of 3.2 million metric tons (FAOSTAT, 2004). Yields are considered to be low at an estimated 851 kg/ha; however, the crop is generally produced on marginal lands in semi-arid environments without the benefit of irrigation and provides nutritious food to local populations. In developed countries, such as Canada, Australia, and the U.S., the crop is a highly valued export commodity. Breeding programs have been established rather recently, as compared to other important food crops, and the primary objectives have been to improve disease resistance, reduce seed and pod shatter, and increase yields. The wild species of *Lens* have been collected and have been used in genetic research to determine species relationships and are a source of genes for crop improvement. The origin, species relationships, genetics, and breeding of lentil are reviewed.

8.2 ORIGIN

It is commonly believed that lentil originated in the Near East arc and Asia Minor (Ladizinsky, 1979). Based on karyotype similarities, and the nearly 100% fertility of hybrids and nearly normal chromosome pairing in meiosis, the progenitor species is considered to be *L. orientalis* (Ladizinsky et al., 1984). Domestication of lentil appeared to depend on development and selection of indehiscent types, including resistance to pod shatter and pod dehiscence, and the loss of seed dormancy through selection. The latter is believed to be a defining event that was critical to domestication. The tendency toward nonshattering pods was also a necessary prerequisite for domestication. Various conclusions concerning the region of domestication of lentil and its distribution are based on the appearance of lentil in early Neolithic settlements that date back to 7,000–6,000 BC (Helbaek, 1959) [A thorough review of the origin of lentil can be found in Ladizinsky (1979)]. From its origin in the Middle East, lentil cultivation spread east through Asia to the Indian subcontinent. The crop also spread into Southern and Central Europe as well as to North Africa. During the times of exploration of the New World, lentil was introduced to South America. Later, immigrants from Central Europe introduced it to North America. Production began in Australia in the late 1900s.

8.3 GERMPLASM COLLECTION, MAINTENANCE, EVALUATION, AND DISSEMINATION

By far the largest germplasm collection of lentil is maintained by the International Center for Agricultural Research in the Dry Areas (ICARDA) located in Aleppo, Syria. The ICARDA collection lists more than 5,000 accessions from 53 different countries and is available to bona fide researchers on request. The USDA-ARS Regional Plant Introduction Station located in Pullman, Washington, maintains a collection of more than 3,500 accessions of cultivated and wild species. National programs in Algeria, Canada, Chile, Ethiopia, Greece, India, Iraq, Sudan, Syria, Australia, and Turkey also maintain germplasm collections of lentil. Most of the accessions held at the gene banks in India and Turkey originated from their respective countries. Some of these collections, along with almost half of the USDA-ARS and IARI collections, are duplicated in the ICARDA collection held in Aleppo, Syria.

The majority of the germplasm collections comprise landraces of the cultivated species (*Lens culinaris* Medik) and relatively few accessions that represent cultivars from breeding programs. The variation within landrace accessions is considerable, and those accessions have been employed in pure-line selection, which has led to the release of improved cultivars. Reflecting the relative difficulty of collection and maintenance of wild *Lens*, the number of accessions of the wild species is rather small. The wild species accessions held in germplasm collections at USDA-ARS, ICARDA, and in Turkey include accessions of *L. orientalis* (Boiss.), *L. nigricans* (M. Bieb.), *L. ervoides* (Brign.), and *L. odemensis* Lad. By far, ICARDA has the largest collection of wild *Lens* germplasm, followed by the USDA-ARS wild *Lens* collection of more than 150

accessions (Muehlbauer et al., 1996). Variation in the characters, such as grain and straw yield, 100 seed weight, days to flowering, days to maturity, seed number per pod, plant height, pod number per peduncle, and resistance to various biotic and abiotic stresses has been shown to be present in the collections. There is also a significant variation for biomass and residue amounts that have been identified for use in breeding programs (Kusmenoglu and Muehlbauer, 1998a, 1998b; Tullu et al., 2001). The world collections held at the major centers are not considered to represent the entire range of variability available within the genus, and additional collection in regions underrepresented in germplasm collections has been proposed. Additional collection of lentil germplasm within and outside the presumed center of origin is still needed and is expected to yield alleles for tolerance to environmental extremes (Muehlbauer et al., 1985).

Many of the accessions in the germplasm collections originated from marketplaces and without a sampling strategy. It is therefore not entirely clear as to the origin of the accessions and if they were actually produced anywhere near the marketplace where they were collected. Another consideration for collections is that many of the accessions do not have proper documentation about the site of collection, other than country.

Germplasm accessions are maintained in duplicates to reduce the risk of their loss. After collection, the accessions are grown for three main purposes: 1) seed regeneration, 2) evaluation, and 3) maintenance of genetic structure (Solh and Erskine, 1981). Regeneration and recycling of the material are made in environments similar to the original collection site to avoid natural selection. Seeds from regeneration then are used for evaluation, distribution, and are held in longterm storage. Evaluation of lentil germplasm collections has revealed valuable traits that impact future breeding strategies (Erskine and Chowdhury, 1986; Erskine and Adham, 1989).

8.4 TAXONOMY

According to Muehlbauer et al. (1980), "The cultivated lentil, *Lens culinaris*, belongs to the Order Rosales, Sub-order Rosineae, Family Fabaceae (Leguminosae), and Sub-family Papilionaceae. Within the Papilionaceae, *Lens* holds an intermediate position between *Vicia* and *Lathyrus*, but appears to be closer to *Vicia*." The Viceae tribe comprises *Lens* Miller, *Vicia* L., *Pisum* L., *Lathyrus* L., and *Vavilovia* A. Fed. (Kupicha, 1981). All genera have cultivated edible legume species except *Vavilovia*. Contradictory interpretations of the species identifications and relationships have been reported in *Lens*. Within the Vicieae tribe, *Lens* holds an intermediate position between *Vicia* and *Lathyrus* but appears to be closer to *Vicia* sec. *Ervum* (Davis and Plitmann, 1970).

The genus *Lens* comprises seven taxa in four species (Ferguson and Erskine, 2001; Ferguson et al., 2000):

L. culinaris Medikus
subsp. *culinaris*
subsp. *orientalis* (Boiss.) Ponert
subsp. *tomentosus* (Ladizinsky) Ferguson, Maxted, van Slageren & Robertson

subsp. *odemensis* (Ladizinsky) Ferguson, Maxted, van Slageren & Robertson

L. ervoides (Brign.) Grande

L. nigricans (M. Bieb.) Godron

L. lamottei Czefr

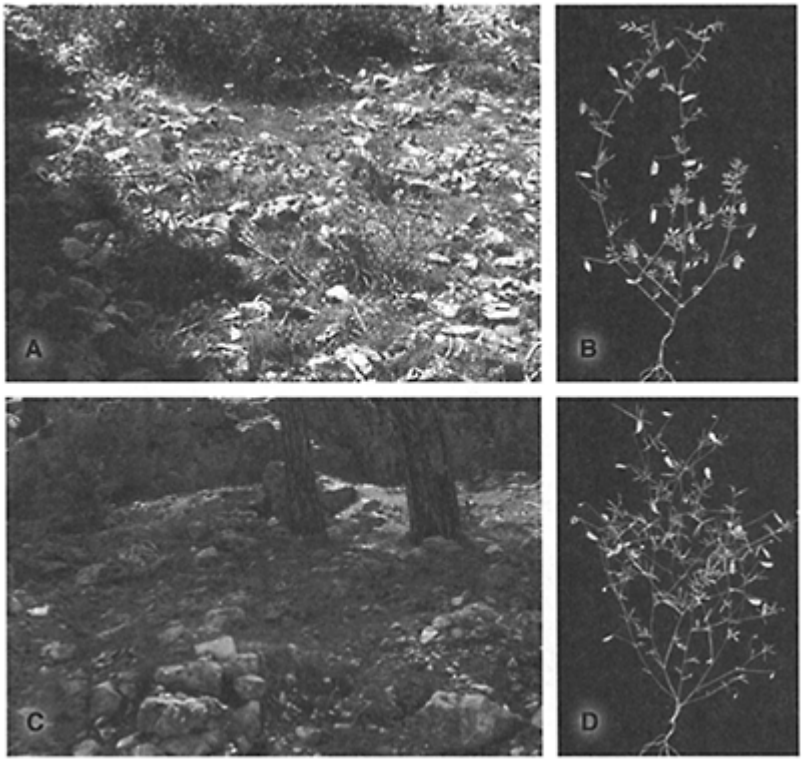

Figure 8.1 Habitat (A) and a typical plant (B) of *L. orientalis* and a habitat (C) and typical plant (D) of *L. ervoides*.

The typical habitat of wild *Lens* species can be characterized by rocky-stony, and shallow calcarious soils with predominantly annual legumes and grasses (Figure 8.1). The habitat of the progenitor species, *L. orientalis* (Figure 8.1A), is mostly rocky, with very little soil, and the small plants (Figure 8.1B) are weakly branched, with relatively long peduncles. *L. ervoides* is often found on the edges of pine forests, where they are able to grow among the pine needles (Figure 8.1C). The plants of *L. ervoides* are characterized by weakly upright stems, with leaves that usually have 2 to 3 leaflet pairs (Figure 8.1D). Stipule shape is used to differentiate *L. orientalis* from *L. nigrican*, with the former having lanceolate stipules, while the latter has either hastate or semi-hastate stipules.

From the standpoint of crossability for use in breeding, the *Lens* species can be divided into two groups (Ladizinsky, 1999): *L. culinaris-L. odemensis* and *L. ervoides-L. nigricans,* which, for convenience, can be assigned to primary, secondary, and tertiary gene pools (Table 8.1). Crosses between members of different groups fail because of hybrid embryo abortion; however, embryo rescue can be used successfully to obtain viable hybrids between groups (Ladizinsky et al., 1985). *Lens orientalis* is generally accepted as being the progenitor of the cultivated lentil *L. culinaris*. *Lens odemensis* is often considered to be in the primary gene pool, based on crossability with the cultivated species; however, there is a great deal of sterility in the progenies (Muehlbauer, 2004), apparently caused by chromosomal rearrangements. The secondary gene pool includes *L. nigricans* and *L. ervoides. Lens ervoides* is crossable to *L. culinaris,* but embryo rescue is needed to recover

Table 8.1 The Gene Pools of Lentil

Primary Gene Pool of *Lens culinaris*	**Secondary Gene Pool**	**Tertiary Gene Pool**
subsp. *culinaris*	*L. ervoides*	*L. lamottei*
subsp. *orientalis*	*L. nigricans*	*L. tomentosus*
subsp. *odemensis*		

the hybrids (Ladizinsky et al., 1985). *Lens nigricans* is also crossable to the cultigen by this procedure. Recently, two new species of *Lens* were identified and described (van Oss et al., 1997). The two new species, *L. lamottei* and *L. tomentosus,* have been identified, and the new classification as independent species is supported by cpDNA restriction patterns (van Oss et al., 1997). *Lens tomentosus* resembles *L. culinaris* except that the pods are tomentose, and there are apparent differences in the karyotype with these latter species having a smaller satellited chromosome that is asymmetrical with a minute satellite. *Lens tomentosus* is reproductively isolated from all other *Lens* species (Ladizinsky, 1997). These latter species are reportedly reproductively isolated from the other *Lens* species and, therefore, might best be placed in a tertiary gene pool.

All *Lens* species share a common chromosome number of 2n=2x=14 chromosomes. However, chromosomal rearrangements play an important role in reproductive isolation between the species. The karyotype of the cultivated *L. culinaris* consists of three pairs of metacentric or submetacentric chromosomes, three pairs of acrocentric chromosomes, and one satellited pair (Ladizinsky, 1979; Slinkard, 1985). The secondary constriction of the satellited pair with a large satellite is close to the centromere in the karyotypes of all of the *Lens* species except *L. tomentosus,* which has a small satellite (van Oss et al., 1997).

8.4.1 Genetics

Classical genetic information on lentil is quite limited and based mostly on morphological traits (Table 8.2). The genetics of lentil was reviewed by Muehlbauer et al. (1996).

8.4.2 Genomics

Genome size of lentil is similar to that of pea—about 4.2 to 4.4 billion base pairs. The genetic linkage map of lentil is still quite rudimentary. A cross between *L. culinaris* and *L. ervoides* was used to develop a map of the genome. However, that cross required embryo culture and had considerable sterility and segregation distortion in the F_2 (Ladizinsky et al., 1985). The *L. culinaris* ×*L. ervoides* cross proved to be especially useful because of a large number of segregating loci that could be used to develop a linkage map. The lentil map was further improved using populations of recombinant inbred lines (Eujayl et al., 1998; Tahir and Muehlbauer, 1994; Tahir et al., 1994). When compared to the pea genetic linkage map, the lentil map had numerous conserved regions (Weeden et al., 1992). Comparison of the lentil map with that of chickpea also revealed conserved regions (Simon and Muehlbauer, 1997); however, the amount of synteny was considerably less for chickpea as compared with pea.

There are no aneuploid series available in lentil for use in mapping experiments. However, an ancient translocation in *Lens* was mapped by using an interspecific cross of *L. culinaris*×*L. orientalis* (Tadmore et al., 1987). In that cross, five linkage groups were mapped, and the reciprocal translocation breakpoint was identified, which differentiated the parents.

The current genetic map of lentil was initially based on linkages between isozyme loci and genes for morphological traits. The first maps of the lentil genome consisted of a small number of markers, mostly isozymes (Weeden et al., 1992). Two linkage groups of isozyme loci and morphological traits were reported in F_2 populations derived from *L. culinaris*×*L. orientalis* crosses (Zamir and Ladizinsky, 1984). The map was extended to include a translocation breakpoint reported by Tadmor et al. (1987) and later to include restriction fragment length polymorphisms (RFLPs). The RFLPs were mapped in lentil using F_2 populations from crosses of *L. culinaris*×*L. orientalis* (Havey and Muehlbauer, 1989). A more comprehensive map of the lentil genome was reported by Eujayl et al. (1998) and consisted of 177 markers, including 89 random amplified polymorphic DNAs (RAPDs), 79 amplified fragment length polymorphisms (AFLPs), 6 RFLPs, and 3 morphological markers (Figure 8.2). A map of mostly dominant type markers consisting of 56 RAPD, 106 inter-simple sequence repeat (ISSR), and 94 AFLP markers was used for a quantitative trait loci analysis of winter hardiness (Kahraman et al., 2004). These maps, with a wide range of marker types, provide a basis for development of a consensus linkage map of the lentil genome.

Table 8.2 Morphological and Disease Resistance Genes in Lentil

Gene Symbol	Trait	Reference
Fn	Number of flowers per inflorescence	Gill and Malhotra, 1980
Gh	Plant growth habit	Ladizinsky, 1979b
Gs	Epicotyl color	Ladizinsky, 1979b
I	Cotyledon color	Slinkard, 1978
O	Cotyledon color (synonymous with *Yc*)	Singh, 1978
Ggc	Grey seed coat ground color	Vandenberg and Slinkard, 1990
Tgc	Tan seed coat ground color	Vandenberg and Slinkard, 1990
P	Flower color	Lal and Srivastava, 1975
Pi	Pod indehiscence	Ladizinsky, 1979
Sbv	Resistance to pea seedborne mosaic virus	Haddad et al., 1978
Scp	Seed coat spotting	Ladizinsky, 1979
V	Flower color	Lal and Srivastava, 1975
		Wilson and Hudson, 1978
W	Flower color	Wilson and Hudson, 1978
Yc	Cotyledon color	Slinkard, 1978
Glp	Glabrous pod	Vandenberg and Slinkard, 1989
Grp	Green pod color	Vandenberg and Slinkard, 1989
Tnl	Tendrilless leaf	Vandenberg and Slinkard, 1989
Chl	Chlorina chlorophyll mutant	Vandenberg and Slinkard, 1989
Ral_1, Ral_2, Ral_3	Resistance to Ascochyta blight	Tay, 1989

Source: Modified from Muehlbauer et al., 1996.

8.5 GERMPLASM ENHANCEMENT

8.5.1 Conventional Breeding

Collection and introduction was initially the procedure for lentil crop improvement throughout the world. That approach rapidly gave way to hybridization and selection over the past 40 years, and now the primary method of breeding appears to be through bulk population or some modification of that method. Single-seed descent, pedigree selection,

mutation breeding, and polyploid breeding have also been employed for lentil crop improvement.

8.5.1.1 Pure-Line Selection

Mass selection or pure-line selection within landraces has been successful in many cases toward improving uniformity and consistency of performance. Landraces represent genetic material that has been subjected to biotic and abiotic stresses over an extended time period, and consequently, the genes that are present or have been selected provide valuable starting material for a lentil crop improvement program. The stability of selections from landraces may be a consequence of the

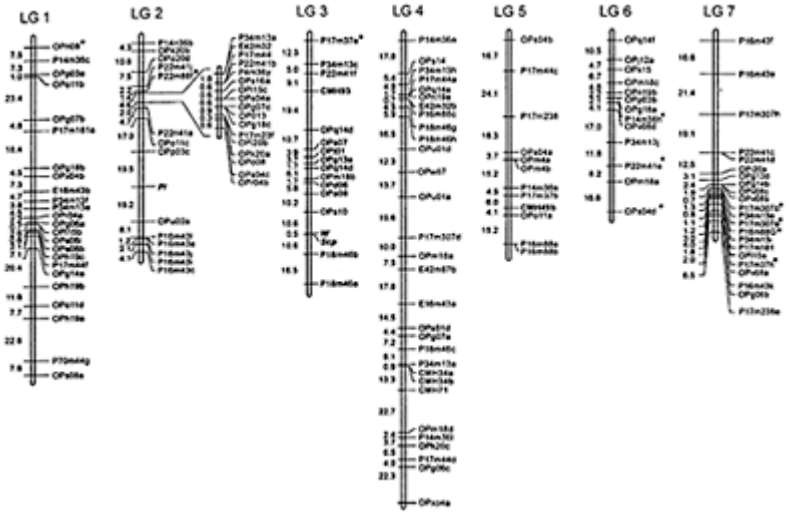

Figure 8.2 A map of the lentil genome (from Eujayl et al., 1998).

highly inbred nature of lentil. Wilson and Law (1972) showed that there was less than 1% or no outcrossing under field conditions in an experiment where outcrossing was estimated using cotyledon color as a genetic marker. Pure-line selection was used to develop cultivars such as ‘Tekoa’ (Wilson et al., 1971), ‘Chilean 78’ (Muehlbauer et al., 1981), and ‘Laird’ (Slinkard, 1978), which have had a major impact on lentil production in North America. An improved cultivar, ‘Araucana-INIA’, released in Chile, was developed by mass-selection from N-1284, a Chilean germplasm accession (Tay et al., 1981). In India, ‘Pant-L-406’ was selected from the germplasm accession P-495 for improved yield and resistance to rust and wilt (Pandya et al., 1980). The focus of lentil improvement programs, while initially centered on selection within landraces and introduced germplasm, gave way to selection primarily within segregating populations from crosses of promising germplasm accessions with locally adapted material.

8.5.1.2 Crossing Procedures

Crossing of lentil is similar to other grain legume crops but is somewhat hampered by the relatively small size of the flowers and their delicate nature. A complete description of crossing techniques in lentil can be found in Muehlbauer et al. (1980, 1996). Briefly, the flowers are cleistogamous and naturally self-pollinating, with an extremely low percentage of natural outcrossing. Crossing in the morning is generally more successful when compared to other times of the day. In making crosses, flowers are chosen that are close to anthesis. At that stage, the wings and standard are unopened and the anthers have not dehisced. A good indication of the proper stage of the female flower for crossing is the length of the sepals relative to the length of the petals. Female flowers are in the prime stage for crossing when the petals are 3/4 the length of the sepals. At that stage, the flower of the female parent is opened with forceps and the 10 anthers carefully removed. Male parent flowers are chosen when the petals have just begun to open. Pollen from the male parent is then gently applied to the stigma immediately after emasculation of the female flower. After pollination, the flower petals are returned to their original position to protect the stigma and developing ovary. Identification tags are then attached to the nodal section of the stem at the point where the peduncle originates. Some breeding programs use color-coded strings to identify crossed flowers and to reduce the time required for identification. The pods with crossed seed are harvested at maturity. The most commonly used genetic marker to identify successful crosses is cotyledon color, which is expressed in the crossed seed. However, cotyledon color is not particularly useful when crossing accessions of the same color. Alternatives include the use of co-dominant molecular markers.

8.5.1.3 Bulk Population Breeding

The bulk population method or some modification thereof is commonly used for lentil breeding. In most programs the populations from crosses are advanced to the F_4, where selection for plant type, earliness, plant height, seed type, or other traits is made in the progenies. The selected plants are then evaluated in single plant rows and advanced to preliminary trials, depending on desirable traits often including disease resistance. After several years of testing, promising lines are released either as improved germplasm or as improved cultivars.

8.5.1.4 Other Breeding Procedures

Backcross breeding has been used in lentil breeding to introduce simply inherited traits into well-adapted and useful cultivars. Pure-line selection is not often practiced, probably because of the time-consuming nature of the method and the difficulties of selection in densely planted breeding lines.

8.5.2 Wide Crossing

Introgression of economically valuable traits through wide crosses holds promise for a number of important traits, particularly for tolerance to abiotic and biotic stresses.

Nevertheless, the use of exotic germplasm for lentil improvement has been limited. In a comprehensive evaluation of wild lentil accessions at ICARDA, significant variation for tolerance to cold temperatures was found in *L. orientalis*.

8.5.3 Mutation Breeding

Mutation breeding may have merit for simply inherited traits and where a suitable and efficient screening procedure is available to identify useful mutants from sufficiently large populations of mutagenized material. There are virtually no reports of mutation breeding of lentils. However, there have been unconfirmed reports of mutation treatments being applied and attempts to develop germplasm with tolerance to herbicides.

8.5.4 Development of Hybrids Using a Cytoplasmic Male Sterile (CMS) System

Hybrid lentil cultivars are not feasible at the present time because the small cleistogamous flowers are virtually 100% self-pollinating, and the pollen is not wind borne. However, in crosses involving wild and cultivated forms, we found what appeared to be a gametophytic cytoplasmic male sterility system composed of sterile (S) and normal (N) cytoplasms, and maintainer (ms) and restorer (Ms) genes (Muehlbauer and Ladizinsky, 2004). A survey of cytoplasmic types in lentil indicated a complex system with at least three cytoplasms that were tentatively designated as S, and S_2 (both sterile), and a single restorer gene with three alleles (Ms1, Ms2, and Ms). A field trial of fully sterile backcross-derived plants resulted in a very low percentage (0.7%) of outcrossed seed. That amount of outcrossing was not sufficient to justify development of hybrid cultivars, but the sterility may be useful in lentil breeding programs.

8.5.5 Genetic Transformation

Genetic transformation of lentil has been accomplished through co-cultivation with *Agrobacterium tumefaciens*. The gene of interest is inserted into the T-DNA and transferred to the lentil genome. This procedure has been successful with other legume species such as *Pisum, Cicer,* and *Lupinus* (Higgins, personal communication, 2004).

Genetic transformation of lentil has been accomplished through co-cultivation with *Agrobacterium tumefaciens*, electroporation, and biolistic particle bombardment. Co-cultivation with *A. tumefaciens* makes use of the bacteria's natural mechanism to transfer DNA (T-DNA) to plant cells. The T-DNA of *A. tumefaciens* has been engineered to remove many of the virulence genes, eliminating the potential to cause disease. Specific gene(s) of interest are then engineered into the T-DNA for transfer to the plant cell during co-cultivation and incorporated into the nuclear genome. Electroporation and biolistics artificially disrupt cell wall integrity to allow DNA entry and subsequent incorporation of the gene in the nuclear genome. Application of particle bombardment as a means of transformation in lentil is a relatively new approach. However, Gulati and McHughen (2003) reported positive preliminary results using the cotyledonary node as explant and chlorsulfuron resistance as the selectable marker.

Transformation efficiency is relatively low (approximately 2%) among the grain legumes and is dependent on plant genotype, *Agrobacterium* strain, and explant. Despite differences in efficiency, several genotypes have been successfully transformed and three strains of *Agrobacterium* have been used successfully: EHA 105, EHA 101, and C58. Several explants including shoot apex, epicotyl, root, cotyledonary node, and longitudinal slices of the embroyonic axis have also been used. Selection of transformants has been accomplished through the use of either antibiotic (hygromycin) or herbicide (basta and chlorsulfuron) resistance (Gulati and McHughen, 2003).

Genetic transformation has many benefits and has the potential to change crop production worldwide; however, transformation of grain legumes, including lentil, suffers low efficiency and a long regeneration and selection period. Improvements in the procedure are needed to optimize production of transformants and will require a systematic and focused approach.

8.6 SUMMARY

Lentil is a world crop of importance in semi-arid regions of the world, where it is grown in rotations that include cereals. Germplasm for use in breeding programs is held at a number of centers throughout the world and is readily available. Lentil originated in the Middle East, where it co-evolved with wheat and barley. Related wild species of *Lens* have been collected and are maintained in the germplasm collections, and are a source of exotic genes for improvement of the cultivated species. Lentil genomics is still in the rudimentary stages; however, linkage maps have been developed, and important genes have been located including those for seed and plant traits, resistance to Fusarium wilt, and tolerance to winter injury. Lentil improvement programs have been successful in the development and release of improved cultivars, which are currently being grown over a wide area. Future improvement of lentil may depend on marker-assisted selection for traits of interest and the introgression of genes from the wild species to broaden the genetic base of cultivated types.

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CHAPTER 9
Lupin

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9.1 INTRODUCTION

Lupinus L. is known in agriculture primarily for its high protein in seed, its ability to fix nitrogen and to grow on soils of low fertility, and its suitability in crop rotations with cereals and oilseeds. It is also known as a horticultural plant, with some of the species having long racemes of attractive and, often, scented flowers. *Lupinus* is a large genus and one of the most widespread, with a rich diversity of species that are divided into two major groups—the Mediterranean/north and east African "Old World" species, and the North and South American "New World" group comprising the greatest species numbers. Parallel indigenous domesticates occur in the Mediterranean and the Andes regions; however, compared with nearly all other food crops, lupins have only recently been the focus of modern crop breeding. Wild lupins are distributed across climatic ranges from subarctic Alaska; Mediterranean and semidesert climates; highland and mountain regions of east Africa, Mexico, the Andes; and High Rockies; the warm temperate climates of the southeastern U.S.; and subtropical regions of eastern South America (Gladstones, 1998). They include simple and compound leaved, herbaceous annual, and herbaceous to shrubby perennial plant types, with the Old World group having only annual species. Generally, lupins are plants of open and well-lit habitats and do not tolerate shading (Gladstones, 1998). The species that have achieved modern crop status are *L. angustifolius, L. albus,* and *L. luteus* from the Old World. *Lupinus cosentinii* Guss. has also been domesticated, although it is not used widely. The New World species *L. mutabilis* has been domesticated but has yet to be used on a large scale as a crop plant. Each of these species, however, possesses useful plant adaptations and seed quality attributes that make the genus a valuable resource for farming practice, production, and use in established feed and emerging food industries. The scope of this chapter is to provide a brief history of the development of domesticated lupins, and a current review of lupin taxonomy, genetic resources, and their use in traditional breeding and involvement of biotechnology in lupin crop improvement.

9.2 HISTORY AND CURRENT POSITION OF LUPINS IN WORLD AGRICULTURE

In 1954, while gold mining at Miller Creek in the Yukon Territory, Harold Schmidt found bones of collared lemmings and remnants of their nests buried 3 to 6 m in frozen silt. With these remains, which were estimated by carbon dating to be at least 10,000 years old, were seeds of *Lupinus arcticus*. Their cold requirement having been satisfactorily fulfilled, a sample of the seeds germinated within 48 hours in the laboratory and developed into healthy plants (Porsild et al., 1967). The historical emergence of the use of lupins by humans, however, is not recorded until after the Pleistocene age. Archaeological evidence has established that at least two of the grain legumes, pea and lentil, played a role with wheat in settled agriculture in the earliest Neolithic sites of the Middle East from 7500 to 7000 BC. Seed of lupins were not recorded for these sites, but their cultivation is believed to date from about 2000 to 1000 BC or earlier in the Mediterranean basin, Egypt, and the central Andean region of South America (Zhukovsky, 1929; von Sengbusch, 1953; Smartt and Hymowitz, 1985). The earliest archaeological reports mentioning lupins are from the 12th dynasty of Egyptian pharaohs (approximately 2000 BC). Tombs from this period contained seeds of lupins (Zhukovsky, 1929). The Latin *Lupinus* means "wolf bean" and implies the plant's ability to grow on rough soils in wild places. Early Greek and Roman writers, such as Theophrastus (372–288 BC), Varro (116–27 BC), and Columella, mention the ability of lupins to grow on and improve poor soils. Varro stated that lupins, while they were still vegetative, were ploughed instead of manure into poor soil. Virgil (70–19 BC) specified lupins as a compatible rotation crop to wheat. Cato the Censor's *De agri cultura*, the oldest Roman agricultural treatise, says "lupin culture will prosper in soils ferruginous or brittle, hard, strong or sandy, provided they are moist." Pliny the Naturalist (AD 23–79) also affirmed the hardiness of the plant by stating that the sowing of lupins was easier than that of any other leguminous seed, requiring "no cultivation"—and that it "costs no expenditure at all" (Rackham, 1971). That lupins were used not only in rotation but as fodder and as human food in adverse times is recorded by Pliny and also by Columella in the first century AD. It was recorded by Pliny that when the Greek painter Protogenes painted his Ialysus in the Temple of Peace in Rome he only consumed soaked lupins to sustain his hunger and thirst and avoided blunting his sensibilities by too luxurious a diet. A special vessel, called a *labrum lupiniarum*, was used by many rural households for leaching the water-soluble alkaloids from the seeds (Hanelt, 1960). Hippocrates of Cos (400–356 BC), Dioscorides in a pharmacological text, and various authors mentioned by Pliny, all stress the positive aspects in lupins, ranging from their medicinal properties (e.g., as an anthelmintic) to their use in cosmetics (e.g., cooked porridge of lupin meal as a skin lotion).

The early primitive domestication and historical cultivation of *L. albus* L. is covered by Knapp (1931), Hanelt (1960), and Hondelmann (1984). It was used by ancient Egyptians, Greeks, and Romans for soil improvement and to precede cereals in crop rotation. It was also used for stock feed or human consumption after boiling or steeping. The debittered seeds of *L. albus* are still a significant snack food in several countries and islands in the Mediterranean region. Many of the other grain legume crops had become widely distributed from their centers of diversity before the period of the Roman Empire,

but it was not until about 200 years ago that *L. albus* spread from the Mediterranean region to Germany and Poland and then both *L. luteus* L. and *L. angustifolius* L. to northwest Europe to be used in the much needed role of soil fertility improvement on poor agricultural lands. In 1780, King Frederick II of Prussia personally requested seed of *L. albus* from Italy for soil improvement in northern Germany (Hondelmann, 1984). Northern European efforts with *L. albus*, however, were not successful because the soils were too poor and the genotypes flowered too late for the region's short summer growing season. Its cultivation in areas of summer growing seasons was confined more to the warmer climates and more fertile soils of eastern central Europe and Ukraine (Gladstones, 1998). New stocks of *L. albus* were introduced from southern France into Germany with greater success in 1817 (Hanelt, 1960). Cultivation of *L. albus* has continued up until the present time on lighter and noncalcareous soils in southern Italy, western Spain and Portugal, the Nile Valley regions to Ethiopia, parts of the Middle East, the western Caucasus Mountain region bordering the Black Sea and France, and central and southeast Europe (Hanelt, 1960; Gladstones, 1970).

Cultivation of *L. luteus* was probably long established in Spain and Portugal, North Africa, and Madeira and is today still used in those regions. Its cultivation in northern Europe as an ornamental occurred at least as early as the sixteenth century. It was when *L. albus* had not performed well in northern Europe that *L. luteus* was experimented with and, by the 1860s, had become an integral part of agriculture on the acid, sandy soils of the Baltic coastal regions for forage and green manuring. As a result of growing yellow lupins, yields of potato and rye doubled (Hackbarth and Husfeld, 1939; Hackbarth and Troll, 1960; Maisurjan and Atabiekova, 1974). Despite the outbreaks of lupinosis in sheep grazing on the stubbles (Gladstones, 1998) and probably poisoning of animals ingesting alkaloids (Kubok, 1988), lupins were used extensively in Germany from that period onward. In the middle of the nineteenth century, lupin spread from Germany to Poland, where it was grown to improve sandy soils. By the end of the nineteenth century, there were 500,000 ha sown to lupins in Germany (Gladstones, 1998). By the early twentieth century, bitter *L. luteus* was grown in Russia and northern Ukraine on acid podsolic soils (Hondelmann, 2000). In addition to broadacre agricultural use, the striking golden yellow, scented flowers of *L. luteus* had meant it became popular as an ornamental in the seventeenth and eighteenth centuries in England (Parkinson, 1629; Curtis, 1791) and in Russia around the early 1800s (Kurlovich, 2002a). *L. pilosus* L. was another Old World species that was brought with *L. luteus* by early horticulturalists (Cowling et al., 1998a).

Lupinus angustifolius was probably the "wild" lupin referred to by classical authors as *L. varius*, a name coined by eighteenth- and nineteenth-century writers (Gladstones, 1970). This is the most common and widespread of the Old World lupins in its natural or near-natural state in the Mediterranean region. It was not domesticated in early times but was spread by early civilizations around the Mediterranean region (Gladstones, 1974, 1998). *Lupinus angustifolius* genotypes, which probably originated from Spain, became established in northern Europe in the nineteenth century (Hanelt, 1960; Hondelmann, 2000). By the early nineteenth century, lupins were growing in southwest France and Germany and by the mid-nineteenth century in Suffolk, England, for sheep feed (Hanelt, 1960) and they were heavily relied upon for the Saxony merino wool industry. *L. angustifolius* had to have an advantage over *L. albus* in that it could tolerate less fertile

and more acidic soils and was less prone to frost (Gladstones, 1998). By the early twentieth century, bitter *L. angustifolius* was grown with *L. luteus* in Russia and northern Ukraine. After the turn of the nineteenth century, however, lupin cultivation fell, due to the introduction of cheap nitrogen fertilizers, and, in the more advanced economies, rising land values favored continuous nonlegume cash crops (Gladstones, 1998). There was also the decline in the Saxony wool industry because of competing imports.

The First World War provided the impetus for renewed interest in lupins as a source of vegetable protein. Researchers in Germany devised processes for de-bittering the seeds, and the potential of lupins for food and other uses was demonstrated in the famous "lupin dinner" of the 1918 Association of Applied Botany in Germany (Hondelmann, 1984). After the war, the first modern breeding of lupins began with private breeders in Germany and Poland. The first aims were for more reliable ripening in the northern European summer. Types having greater plant vigor and earlier maturity were the products of these early programs (Hanelt, 1960). The next and most important development was the selection of low-alkaloid lupins, following a suggestion by Professor Erwin Baur, the director of the Kaiser Wilhelm Institute for Genetic Research in Berlin. After adapting methods for lupin alkaloid analysis developed in Russia by D.N.Pryanishnikov, a researcher at the Kaiser Wilhelm Institute, Reinhold von Sengbusch (1898–1986) in 1928–1929, selected the first lowalkaloid natural mutant genotypes of *L. luteus* and *L. angustifolius* and then, shortly afterward, in *L. albus, L. mutabilis* Sweet, and *L. perennis* (von Sengbusch, 1942). The cultivars arising from this were Müncheberger Gelbe Grünfutetrsüsslupine St. 8 and Müncheberger Blaue Grünfutter Süsslupine, respectively (Hackbarth and Troll, 1960; Gladstones, 1970). The results and seed material from this work were kept secret until many years later. The new alkaloid genotypes all proved to be conditioned by simple recessive genes and were assumed to be natural mutants (Hackbarth, 1957a, 1957b; Hackbarth and von Sengbusch, 1934). The method for the rapid detection of low alkaloid plants was used independently to obtain selections in *L. angustifolius* and *L. luteus* in Russia under the leadership of N.N.Ivanov at the Institute of Plant Industry (VIR). This work was published for the Russian and wider research community at the time (Fedotov, 1932, 1934; Ivanov et al., 1932, cited by Kurlovich, 2002a; Svirskii, 1934; Hackbarth, 1957a, 1957b). The first yellow lupin cultivar carrying the low alkaloid trait from this work in Russia was cv. Yubileiny. The area sown to lupins rapidly rose in Germany as a result of the development of low alkaloid breeding material. Their use in other countries was discouraged due to patent and secrecy restrictions. Large areas were sown in occupied Hungary and Poland, and after the Second World War, there was an even greater interest in Poland for the crop on poor soils. Once low alkaloid lupin genotypes were available, areas in Russia increased after the Second World War, *L. luteus* was grown in Byelorussia, northern Ukraine, and the Baltic States, while *L. angustifolius* in the central and northern areas because of its shorter maturity time and cold resistance, and *L. albus* in southern Ukraine, the Caucasian coast of the Black Sea (Gladstones, 1970).

Lupinus angustifolius was introduced to New Zealand, Australia, and South Africa only during the first half of the twentieth century. By the 1930s, lupins had become established on the Canterbury Plains in New Zealand for soil improvement and sheep husbandry (Hudson, 1934), and from that source it was introduced into Australia in the 1930s and 1940s, mainly for green manuring in orchards and vineyards. Roles as a green

manure and sheep feed were also found for *L. angustifolius* in the Cape Province of South Africa and the southeast coastal areas of the U.S. (Gladstones, 1970). The rough-seeded lupins of the Old World were probably used in very ancient times for human consumption in north Africa and in the eastern Mediterranean, and this may have contributed to their evolution as large-seeded species over hundreds of thousands of years (Gladstones, 1974, 1998; Plitmann, 1981). Lupins are hardy and generally thrive on lighter, infertile soils. This, together with their ability to volunteer on disturbed sites, would have favored their association with presettled agriculture. The rough-seeded lupins are bitter in vegetative parts, but their seeds have only moderate levels of alkaloids, and direct use may have been possible. Larger seed size and reduced bitterness would have been favored and provided a selection pressure. Following the move to settled agriculture during the Neolithic period, lupin use would have declined when the newly domesticated crops were used.

Lupinus mutabilis, the species that is notable among lupins because of its high protein and oil content, was much more confined in its spread as a crop plant. It is one of several species with its origin and cultivation as a food crop used in the Andean region for thousands of years. That same region is the origin of tomato, corn, cotton, peanut, and several *Phaseolus* crops. Hondelmann (1984) suggested an ancient history of domestication of the species dating back to 600–700 BC. Seed fragments were discovered in Nazca civilization tombs (100–500 AD) in Peru (Antúnez de Mayolo, 1982). When the Spanish arrived at the beginning of the sixteenth century, the native people of the Andes were already cultivating and consuming it on a wide scale. Although bitter, the seeds were soaked in running streams to remove alkaloids. There were indications of inclusion in the diets in the order of 5% around this time. Frequent medicinal uses as seed or leaf ranged from increasing fertility to a coffeelike preparation that also acted as a vermifuge. The seed decoction was used as a stupefacient in fishing or as an insecticide (Antúnez de Mayolo, 1982). It is known as “Chocho” from Columbia through Ecuador to central Peru, “tarwi” in Quecua and “tauri” in Aymara toward Bolivia, “chuchus” (woman’s nipple) in Cochabamba, “ccequla” in Azangaro, and “ullush” in Tarma (Antúnez de Mayolo, 1982; Tapia and Vargas, 1982). Due to the similarity of *L. mutabilis* with the Spanish “Chocho” used for *L. albus*, that name was adopted by the native people for the indigenous species (Antúnez de Mayolo, 1982). At a 1986 conference of the International Lupin Association, the name “Andean lupin” was proposed for international use. This species’ agricultural role was only within its original natural distribution in the Andes, at least until breeding programs began in countries such as Germany in the second half of the twentieth century (e.g., Römer and Jahn-Deesbach, 1986). Cultivation of *L. mutabilis* occurs at altitudes from 2000 to 3400 m and is found as high as 3800 m in the Lake Titicaca region of Peru and Bolivia and high altitude valleys in the south of Bolivia and in Ecuador (Gade, 1975; Blanco, 1982; Planchuelo, 1994). Low-alkaloid plants were identified in *L. mutabilis* in 1942 by von Sengbusch, in 1961 and in 1971 by Brucher, but for various reasons, including the extreme lateness of the parent genotypes used, these lines were lost (von Sengbusch, 1942; Harrison, 1982). Von Baer and Gross (1977) then succeeded in developing fertile low-alkaloid lines from natural mutants, which have been subsequently used in breeding programs. This species has been cultivated in temperate climates in some countries as an ornamental for their

long, multicolored flowers and pleasant scent, but it is still not widely cultivated outside South America.

Several other of the New World species have received attention in agricultural, land rehabilitation, or horticultural contexts. *Lupinus polyphyllus* (sometimes referred to as the Washington lupin) and its hybrid, the "Russell lupin" are cultivated worldwide as ornamental plants (Perry, 1972), as forage in Scandinavia and Iceland (Plarre, 1991; Fremstad and Elven, 2002), and as a pasture in New Zealand (Hill, 1986; Hill and Miller, 1990). The Mexican species *L. mexicanus* (*L. ehrenbergii*, commercially named *L. hartwegii*), has been known as an ornamental plant since the mid-twentieth century (Burkart, 1959). The Russell lupin was bred by horticulturalist George Russell in 1937 as a garden ornamental in England and to mark the coronation of King George VI. With the aim of selecting brighter flower colors, Russell made crosses between north, central, and southern American species, particularly *L. polyphyllus, L. laxiflorus, L. lepidus, L. hartwegii*, and *L. mutabilis*. The parentage of the Russell lupin was thought to be made up of *L. polyphyllus* and *L. arboreus*, and possibly the Alaskan species *L. nootkatensis* (Gorer, 1970). Horticultural breeders still continue to develop even more beautiful forms of lupins based on such species. Several selections of low-alkaloid forms of *L. polyphyllus* were bred in 1932 at the VIR in Russia (Kurlovich, 2002a) and elsewhere (Plarre, 1991; Römer, 1995). *Lupinus subcarnosis*, the Texas or Sand bluebonnet native to eastern Texas and *L. havardii*—the Big Bend bluebonnet, found in the Chihuahuan Desert, are known for their showy flowers. Interest in the development of lesser-known lupin species such as *L. albescens, L. gibertianus*, and *L. magnistipulatus* as ornamentals still continues today (Mackay et al., 1999; Planchuelo and Fuentes, 2004). Other species used for soil conservation or amelioration are *L. albescens, L. aureonitens*, and *L. multiflorus* (Planchuelo, 1994). *Lupinus nootkatensis* has been used in land reclamation in Iceland since it was introduced there from its natural coastal origins in Alaska in the mid-twentieth century. It has colonized areas that have been degraded by extensive removal of native birch forest, which once covered a major part of the land area. This lupin species has been particularly tolerant of high winds, oceanic salt spray, and nitrogenpoor soils (Magnusson et al., 2004).

A valid explanation for the slow development of lupins as a modern crop, despite its use in primitive agriculture for a substantial time, is the innate presence in all of the species of high concentrations of quinolizidine alkaloids in the plant and seed (Petterson, 1998). This group of substances is common in Fabaceae, but is also present in some other unrelated families (Schuette, 1969). Another major factor has been the increased competitiveness of soybean (Williams, 1982; Pate et al., 1985; Williams, 1986). Despite the discovery of low-alkaloid genotypes, development of lupins in European agriculture has still been relatively slow, and they retain a minority position among legumes such as common bean, pea, and faba bean. *Lupinus albus* was introduced into areas considered beyond its Mediterranean basin climatic adaptation range. The resulting problems included unpredictable optimal date of sowing when spring-sown in northerly latitudes, slow and late maturity of pods and seeds, and the highly indeterminate growth habit, producing excessive vegetative growth (Williams, 1982). A restriction (and yet a great advantage in suitable areas) for *L. angustifolius* and *L. luteus* has been their adaptation primarily to acidic and relatively well-drained soils. Areas of lupin cultivation declined in Germany since the Second World War from 32,000 ha to only small plantings currently.

In Poland, significant areas were sown just after the war (almost 200,000 ha) but declined by the mid-1970s, then increased again in the 1980s (to approximately 115,000 ha), followed by declines to an area of 10,000 ha in 2002 (FAO, 2003), Areas of lupins sown in the former USSR increased to a maximum of more than 600,000 ha by the late 1970s but then declined to less than 300,000 ha in the mid 1980s—and further still to an official figure of 14,000 ha for the Russian federation currently. Areas in countries such as Belarus have increased in recent decades, with only 30 ha reported in 1988 to more than 60,000 ha in 1998 (Kuptsov, 2000). Significant areas of lupins were grown in South Africa by the late 1960s (approximately 200,000 ha), but this area also declined to 20,000 ha by the mid-1980s and is currently at an area approximately of 11,000 ha. Other countries with significant plantings are France (13,000 ha), Spain (17,000 ha), Morocco (21,000 ha), and Chile (16,000 ha).

The most spectacular adoption of lupins has been in Australia, which began with a local breeding program based on *L. angustifolius* initiated by Dr. John Gladstones in the late 1950s (Gladstones, 1959; Gladstones, 1960). From zero commercial production just prior to the release of the first Australian cultivar in 1967, the area has increased to a maximum of 1.425 million ha in 1997 and still sits at approximately 930,000 ha (FAO, 2003). With vast areas of generally coarse-textured acid and infertile soils, lupins were perfectly suited particularly to the "wheatbelt" of Western Australia with its Mediterranean climate, and it is this region that has represented 90% of the area sown in Australia. Lupins were introduced just at the end of a period of rapid expansion and clearing of land for agriculture in southwestern Australia. With the subterranean clover-wheat rotation system in decline because of weeds, disease, and reduced pasture seed banks, a new legume with the characteristics of lupin was a welcome solution (Perry et al., 1998). A combination of activities enabled lupins to expand successively each year over three decades. These were a dedicated breeding and evaluation program that proceeded in parallel with agronomic and farming systems development and extension packages; market development; and enthusiastic cooperation by progressive farmers (Perry and Poole, 1975; Walton, 1976; Nelson and Delane, 1991; Nelson, 1994; Nelson and Hawthorne, 2000). Lupins are entering a new phase with prospective high-value markets for the grain as a quality feed, including in aquaculture and the food ingredient industry based on several excellent functional properties of the protein and fiber fractions (Lqari et al., 2002; Sipsas, 2004). In Australia, efforts are now being made to breed lupins with increased levels of protein and sulfur amino acids to match grain quality to specific end-use requirements (Sweetingham et al., 2004).

9.3 TAXONOMY, CENTERS OF DIVERSITY, AND DISTRIBUTION

9.3.1 Taxonomy

The taxonomy of lupins has been dealt with by several authors, often with particular emphases on Old or New World species. These include Gladstones (1974, 1984, 1998), Plitmann (1966, 1981), Plitmann and Heyn (1984), Nowacki and Prus-Glowacki (1971), Dunn (1979, 1984), Planchuelo-Ravelo (1984), Planchuelo and Dunn (1984); Planchuelo

(1985, 1994). The precise number of lupin species is unclear, but there could be up to 600 taxa. The number of published botanical names has increased from the six species published by Linnaeus in 1753, to approximately 1700 species names, published in various floristic and monographic works (Planchuelo, 1999). Until the major taxonomic revision of Gladstones (1974), the taxonomy of the Old World lupins was confused as a result of mistakes from the early years of Linnaean taxonomy. The taxonomy of the New World lupins has proven difficult due to the large number of apparent species and intermediates resulting from outcrossing and a high degree of phenotypic plasticity across their habitats (Planchuelo, 1994).

Lupins are classified taxonomically as order Fabales Bromhead, family Fabaceae Lindley, tribe Genisteae (Adamson) Bentham, genus *Lupinus* L. Despite its broad distribution and relatively variable morphology in the genus, the unity of the genus is unquestioned and the majority of authors agree in assigning the genus to the tribe Genisteae. Some variations to this taxonomic citation, however, do occur. In a systematic review, Bisby (1981) proposed that *Lupinus* be included in the subtribe Lupininae (Hutch.) of the tribe Genisteae (Adanson) Bentham. The use of subfamily names Papilionoideae and Faboideae is also prevalent (Stevens, 2001; NCBI 2004), and family Leguminosae Jussieu is usually considered synonymous with Fabaceae (Stevens, 2001). There is still some dispute about the tribal position of *Lupinus* (Monteiro, 1986; Saint-Martin, 1986; Badr et al., 1994; International Plant Names Index, 2004). For example, one study using chloroplast DNA polymorphisms of 35 legume species proposed that *Lupinus* should not be included in the tribe Genisteae, based on the argument that another genus *Anagyris* (tribe Thermopsideae) appeared to be more closely related to other Genisteae than *Lupinus* (Badr et al., 1994). A more recent study, however (Ainouche and Bayer, 1999), based on internal transcribed spacer sequences (ITS) of nuclear ribosomal DNA using 44 *Lupinus* taxa and five outgroup taxa, clearly supported previous classifications (Polhill, 1976; Bisby, 1981) that the *Lupinus* genus is a strongly monophyletic genus and should be included in tribe Genisteae, but as a distinct lineage [subtribe Lupininae (Hutch.) Bisby] from the Genistinae ("Cytisus-Genista complex"). The results were consistent with serological data (Cristofolini and Feoli Chiapella, 1977; Cristofolini, 1989) and molecular-based phylogenies of the Papilionoideae (Doyle, 1995; Käss and Wink, 1995, 1997a, 1997b; Doyle et al., 1997). That study also placed Crotalaria as sister to the Genisteae and Thermopsis more distantly related to *Lupinus* and sister to the Crotolaria-Genisteae group.

Two geographically separate groups within the genus *Lupinus* are recognized—the New World species, distributed from Alaska to the extreme south of South America at the island of Tierra del Fuego (Argentina and Chile), and the Old World lupins consisting of 12 species distributed in the Mediterranean and North African region. This very diverse and widespread genus occupies habitats from sea level to alpine tundra up to 4000 m altitude. Species vary from annual to perennial, with growth habits from acaulescent or small prostrate to tree-like shrubs reported to be 4.5 m (Dunn, 1984) or even to 8 m high, with woody trunks of 30 cm across such as in *L. jaimehintoniana* B.L. in Mexico (Turner, 1995). The main features that distinguish the genus *Lupinus* are large and numerous flowers on terminal racemes (Figure 9.1); flowers with a deeply cleft calyx, erect standard petal; wings connate at the apex; keel incurved, beaked, and enclosed within the wings; stamens 10, alternately long and basifixed, short and versatile;

ovary sessile; style incurved, glabrous; stigma terminal; pod oblong, more or less compressed, septate between the seeds, valves thick and leathery, dehiscent; cotyledons thick and fleshy (Allen and Allen, 1981). The distinctiveness of the New World and two groups of the Old World lupins has been supported by various authors including those based on seed globulin patterns (Przybylska and Zimniak-Przybylska, 1995; Zimniak-Przybylska and Przybylska, 1997), protein serology (Cristofolini, 1989), and general taxonomic characteristics (Plitmann and Heyn, 1984).

In addition to the geographic division of the Old and New World, lupins can be separated into two groups based on leaf characters, one including species with simple leaves of approximately 26 species and the other with typically palmately compound leaves (Planchuelo, 1994). The other subgeneric division is made on seed testa structure (Plitmann and Heyn, 1984)—seven species that have rough testa (section Scabrispermae) are all distributed in the Old World, while the rest are typically smooth (section Malacospermae) and include the economically important species, *L. angustifolius, L. luteus, L. albus,* and *L. mutabilis.* The rough-seeded micromorphology is characterized by the pluricellular tubercules of the seed coat, which are not present in the smooth-seeded species (Lush and Evans, 1980; Plitmann and Heyn, 1984; Ainouche and Bayer, 2000). Although floral morphology is relatively uniform in the genus, several characters with taxonomic value show a high degree of variation between species (Planchuelo-Ravelo, 1984). Support for the separation of the species into the groupings rough- and smooth-seeded is provided by studies using flavonoids (Williams et al., 1983), isozymes (Wolko and Weeden, 1990b), seed globulin patterns (Przybylska and Zimniak-Przybylska, 1995; Zimniak-Przybylska and Przybylska, 1997), cytology (Plitmann and Pazy, 1984), coat structural comparisons (Heyn and Herrnstadt, 1977), and DNA analysis (Kass and Wink, 1997b; Ainouche and Bayer, 1999) (Figure 9.2).

9.3.1.1 Old World Lupin Species

The generally accepted taxonomy of the Old World lupin species is that of J.S.Gladstones, whose comprehensive studies at several herbariums from 1968 resulted in the publication of revised taxonomy, history, and use of lupins (Gladstones, 1970, 1974). A summary of the botanical descriptions of the Old World lupins are given in Table 9.1, based primarily on Gladstones (1974). The botanical key can be found in Gladstones (1974, 1998). This classification is supported by other studies (Salmanowicz and Przybylska, 1994; Ainouche and Bayer, 1999; Salmanowicz, 1999; Naganowska et al., 2003a) and is highly congruent with recent ITS DNA data (Ainouche and Bayer,

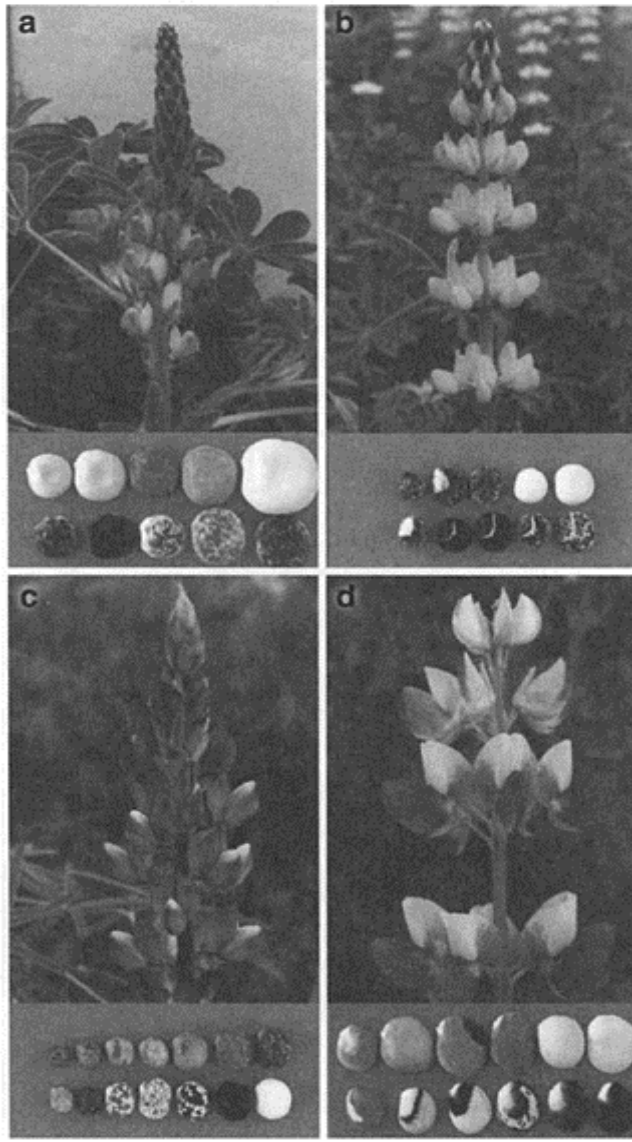

Figure 9.1 (See color insert following page 178) Flowers and seeds of: (a) *Lupinus albus,* (b) *Lupinus luteus,* (c) *Lupinus angustifolius* (wild type flower color shown), and (d) *Lupinus mutabilis.*

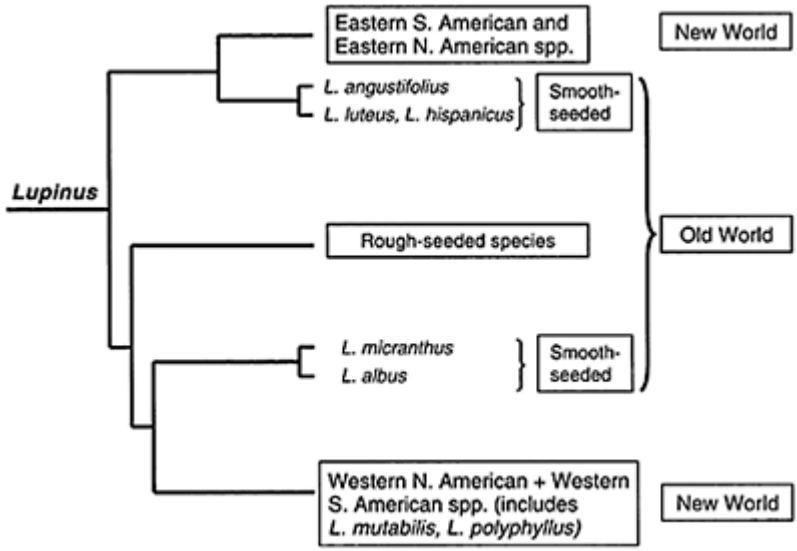

Figure 9.2 Generalized diagram showing phylogenetic relationships among Old and New World Lupinus (from Kass and Wink, 1997b; Ainouche and Bayer, 1999; Merino et al., 2000).

1999). Nowacki and Prus-Glowacki (1971) distinguished five groups of species on serological grounds and these agreed well with morphology and ability to form interspecific hybrids among the Old World group as discussed by Gladstones (1984). Despite showing a range of chromosome numbers, the morphological, serological, genetic, isozyme, and interspecific crossing ability evidence shows that the rough-seeded species from the Old World are very homogeneous (Gladstones, 1974; Plitmann, 1981; Plitmann and Heyn, 1984; Cristofolini, 1989; Wolko and Weeden, 1990a; Carstairs et al., 1992; Gupta et al., 1996) and the most strongly supported clade in the Old World group (Ainouche and Bayer, 1999). In one study based on nuclear DNA contents, the rough-seeded *L. princei* (2n=38) was found to be closer to the smooth-seeded Old World species than the other rough-seeded ones but did not dispute its place among rough-seeded species (Naganowska et al., 2000a). Carstairs et al. (1992) placed the rough-seeded lupins, based on cytogenetic and crossability studies, into three groups: the Princei group *(L. princei)* in equatorial Africa, the Atlanticus group (*L. atlanticus, L. cosentinii*, and *L. digitatus*) in northwestern Africa, and the Pilosus group (*L. pilosus* and *L. palaestinus*) in the eastern Mediterranean. The new species, *Lupinus anatolicus* Swiec., was proposed by Swiecicki et al. (1996) after a single smooth-seeded seed sample was collected from the hills near Efez, Turkey (Swiecicki, 1988a), which looked similar to the roughseeded *L. pilosus*. However, this accession has the same chromosome number as *L.*

pilosus (2n= 42) (Buirchell and Cowling, 1998) and falls within the range of variation of *L. pilosus* reported by other authors (Clements et al., 1996). Additionally, three accessions containing smooth seeds have been found within *L. pilosus* accessions from the Tartus and Homs regions of southern Syria (Buirchell, 1999). Buirchell has made smooth-seeded selections from mutation breeding programs with *L. pilosus* (Cowling et al., 1998a). Closer examination of the seed coat structure, in addition to ITS data, has since suggested close similarities of *L. anatolicus* with the rough-seeded *L. pilosus* (Ainouche and Bayer, 2000). Based on these findings, the differences reported for the variant with respect to seed protein, oil, alkaloid, and isozyme results (Przybylska and Zimniak-Przybylska, 1995; Swiecicki et al., 1996), might not be sufficient to warrant the status of new species.

L. pilosus was the first species of the rough-seeded group to be described within the Old World lupins and Plitmann and Heyn (1984) nominated *L. pilosus* as the Type Species for the Scabrisper-

Table 9.1 Old World Lupins and *L. mutabilis*. Species Names, Synonyms, Common Names, Distribution of Species (with Species Authors), and Chromosome Numbers (2n)

Species	2n	Main Botanical Synonyms	Common Names	Natural Distribution Summary	Domestication Status
			Old World Smooth-Seeded		
L. albus L.		*L. termis* Forsk.	Greek derivatives: termis, turmas, turmus		
		L. varius Gaertn.	Latin derivative: lupino		
		L. sativus Gater.	English: albus lupin, white lupin; French: lupin		
		L. hirsutus Eichw.	German: weisse Lupine; Italian: lupino		
			Spanish and Portuguese derivatives: altramuz, tremocos, entramocos, chochos; Russian:		

			Ijupin belyj; Turkish: aci bakla		
L. albus L. var. *albus*	50	*L. albus* L.	As for *L. albus* L.	Pan Mediterranean cult.	Early cultivation, semi-domestication
		L. albus L. subsp. *albus*			Now domesticated crop
		*L. albus L. va*r. termis (Forsk.) Caruel			
L. albus L. var. *graecus*	50	*L. graecus* Boiss. & Sprun.	English: wild albus lupin	NE Mediterranean wild	Wild
(Boiss. & Sprun.) Gladst.		*L. juvoslavicus* Kazim. & Now.			
		L. vavilovi Atab. & Maiss.			
L. angustifolius L.	40	*L. varius* L.	English: narrow-leafed lupin, blue lupine	Pan-Mediterranean, native and semi-cult.; Australia,	Later cultivation and landrace selection
		L. linifolius Roth.	German: schmalblattrige Oder blaue Lupine	naturalized and cult.; elsewhere cult.	Domesticated crop
		L. reticulatus Desv.			
		L. leucospermus Boiss.			
		L. opsianthus Atab. & Maiss.			
L. luteus L.	52		English: yellow lupin; German: gelbe Lupine; Spanish: altramuz amarillo	W Iberia, pan Mediterranean, native and semi-cult.; elsewhere cult.	Early cultivation and landrace selection, ornamental use; Domesticated crop past 60 years

L. hispanicus Boiss. & Reut. subsp. hispanicus	52			Central and S. Spain; ? Algeria, Greece, Turkey; native	Wild

Table 9.2 Old World Lupins and *L. mutabilis*. Species Names, Synonyms, Common Names, Distribution of Species (with Species Authors), and Chromosome Numbers (2n) (Continued)

Species	2n	Main Botanical Synonyms	Common Names	Natural Distribution Summary	Domestication Status
subsp. *bicolor* (Merino) Gladst.	52	*L. rothmaleri* Klink., *L. hispanicus var. bicolor Merino*		Central, NW Spain, Portugal,? Greece, native	Wild
L. micranthus Guss.	52	L. *hirsutus* L.			Wild; used in classical times as green manure
			Old World Rough-Seeded		
L. *pilosus* Murr.	42	*L. hirsutus* L., *L. varius* L., *L. varius* spp orientalis		NE Mediterranean, native and ? semi-cult.	Early landrace selection for human consumption, ornamental use; Recently domesticated
		L. anatolicus Swiec.			
L. cosentinii Guss.	32	*L. hirsutus* Black, *L. digitatus* Lojac., *L. pilosus* ssp cosentinii, *L. varius*	English: Sandplain lupin, Western Australian blue lupin	W. Mediterranean, Morocco, native: Australia, naturalized	Recently domesticated
L. *digitatus* Forsk.	36	*L. tassilicus* Maire,		Sahara, native	Wild, possible early selection, recently domesticated

					(interspecific crossing)
		L. semiverticillatus Desr.			
L. princei Harms	38			E Africa, native	Wild
L. palaestinus Boiss.	42			SE Mediterranean, native	Wild, possible early human selection
L. atlanticus Gladst.	38		English: Atlas lupin, Moroccan lupin	S Morocco, native	Wild, possible early selection for human consumption, recently domesticated (interspecific crossing)
L. somaliensis Baker	?			E Africa, native	Possibly extinct
			New World Crop Species		
L. mutabilis Sweet.	48		English: Andean lupin, pearl lupin, tarwi; Spanish: chochos; Local languages: tarwi, tauri, chuchus, ccequla, ullush	South America, Andean region	Wild, landrace, and domesticated

Source: From Kazimierski, 1960, 1961; Pazy et al., 1977; Gladstones, 1970, 1974, 1984, 1998; Cowling et al., 1998a; Carstairs et al., 1992; Buirchell, 1999.
Note:?=not certain how recent.

mae. Gladstones (1974; 1998) describes the confusion in the taxonomy and historical synonyms for this species. Some *L. pilosus* accessions have been classified as *L. pilosus* but show some of the characteristics of *L. palaestinus*, including flower appearance, a requirement for tripping to achieve self-pollination and *Bradyrhizobium* compatibility (Gladstones, 1974; Gladstones and Crosbie, 1979; Clements et al., 1996). These types could be intermediates between the two species as reported by Plitmann et al. (1980) and Pazy et al. (1981). *Lupinus palaestinus* has the same chromosome number as *L. pilosus*,

and their ability to experimentally intercross, albeit with considerable genetic barriers, suggests a close relationship between them. DNA contents were also very similar for *L. pilosus* and *L. palaestinus,* compared to *L. cosentinii* and *L. atlanticus* (Ghrabi et al., 1999). Other studies confirming the *L. pilosus/L. palaestinus* relationship are found for ITS data (Ainouche and Bayer, 1999), seed proteins (Salmanowicz and Przybylska, 1994; Salmanowicz, 1999), and interspecific crossing ability (Kazimierski, 1961; Carstairs et al., 1992), but none have suggested combining them as one species.

Lupinus cosentinii (2n=32) has been referred to in the past as *L. cosentini, L. varius,* and *L. digitatus,* but clarification was established by Gladstones (1970, 1974) and through chromosome counts. Moroccan or Tunisian genotypes differ with respect to some morphological characters to those from the northern shores of the Mediterranean and Australian naturalized populations. Despite reports of reduced fertililty in progeny from crosses between the two (Kazimierski, 1964b), it was concluded that subspecific division was not justified (Gladstones, 1984). Based on ITS data, *L. cosentinii* is separated from the *L. pilosus/L. palaestinus* and the *L. digitatus/L. atlanticus* subdivisions but is more closely related to the latter two (Ainouche and Bayer, 1999). This closer relationship is reflected in seed protein studies (Salmanowicz and Przybylska, 1994; Salmanowicz, 1999), which produced the groupings (1) *L. atlanticus,* (2) *L. cosentinii* and *L. digitatus,* and (3) *L. palaestinus* and *L. pilosus.* Cytological and interspecific crossing studies (Carstairs et al., 1992) classified the rough-seeded species as the Atlanticus group (*L. atlanticus, L. digitatus,* and *L. cosentinii*), the Pilosus group *(L. pilosus, L. palaestinus).*

Lupinus digitatus (2n=36, Plitmann and Heyn, 1984) has been referred to as *L. tassilicus* Maire (Gladstones, 1974; Ainouche et al., 1996) due to earlier confusion and grouping of *L. digitatus* with *L. pilosus,* but morphological and chromosome evidence support the synonymy of *L. tassilicus* and *L. digitatus* (Gladstones, 1974; Plitmann and Heyn, 1984; Carstairs et al., 1992). Synonyms for *L. atlanticus* were similarly clarified by Gladstones (1974). *L. atlanticus* has the same chromosome number as *L. princei* (2n=38) (Plitman et al., 1980; Carstairs et al., 1992), but crossing and cytological studies suggest closest genetic affinity to *L. cosentinii* (Roy and Gladstones, 1985, 1988; Carstairs et al., 1992; Gupta et al., 1994, 1996) with some affinity to *L. pilosus* (Gladstones, 1998). The geographically isolated *L. princei* is relatively distinct morphologically and is identified in phylogenetic studies to clearly belong to the rough-seeded group (Käss and Wink, 1997b). Naganowska et al. (2003a) found the nuclear DNA content closer to the smooth-seeded species (very similar to *L. micranthus*), and Carstairs et al. (1992) noted that it had the longest chromosomes among the rough-seeded lupins analyzed. It has failed to produce viable seeds when crossed with other rough-seeded species (Carstairs et al., 1992; Gupta et al., 1996), and this, along with its morphological distinctiveness, supports its status as a separate species. *Lupinus somaliensis* is only known from its Type Specimen plant collected in the nineteenth century, which did not include seeds or pods. Gladstones (1974) noted some relatively distinct morphological differences between it and *L. princei,* and it has maintained its status as a separate rough-seeded lupin that was probably ecologically isolated in the equatorial highlands of Somalia. *Lupinus somaliensis* was tentatively placed in the Princei group by Carstairs et al. (1992). Confirmation of its existence is still pending. Further taxonomic evaluation and exploration appears worthwhile for the rough-seeded lupins because of the smaller

numbers of available accessions, the tendency for ecogeographical isolation among many of these species, and the existence of numerous isolated populations in their habitats in North Africa and the eastern Mediterranean.

The smooth-seeded Mediterranean group are a much less homogeneous, more widely separated group, and have major genetic barriers between most species (Plitmann and Heyn, 1984). Genetic, biochemical, and serological studies support this separation (Nowacki and Jaworski, 1978; Cristofolini, 1989; Wolko and Weeden, 1990b). The smooth-seeded Old World lupins were separated into four distinct sections *(albus, angustifolius, luteus, micranthus),* all monospecific apart from the *L. luteus/L. hispanicus* complex (Nowacki and Prus-Glowacki, 1971; Gladstones, 1984). Its genetic evidence resolves them into two distinct clades, and within these clades, all species are well defined (Ainouche and Bayer, 1999). This data was supported by alkaloid (Nowacki, 1963; Wink et al., 1995), leaf flavonoid (Williams et al., 1983), seed protein (Salmanowicz and Przybylska, 1994; Przybylska and Zimniak-Przybylska, 1995), isozyme data (Wolko and Weeden, 1990b), and other DNA data (Kass and Wink, 1997b). One of the clades defined by Ainouch and Bayer (1999) consisted of three species, *L. angustifolius* (2n=40), *L. luteus,* and *L. hispanicus* (both 2n=52). This indicated an unexpectedly close relationship between the *Angustifoli* and *Lutei* sections described by Nowacki and Prus-Glowacki (1971) and Gladstones (1984), despite them being relatively distinct plants in morphology and cytology. *Lupinus luteus* was proposed as being closer to *L. micranthus,* based on some similar morphological traits and because both have the same chromosome number (Gladstones, 1984). Some support for the connection between *L. luteus* and *L. angustifolius* might also be provided by the existence of a "foveolate" seed coat pattern found in *L. angustifolius* samples from North Africa that were similar to the appearance of *L. luteus* seed coat types (Ainouche and Bayer, 1999).

Lupinus luteus and *L. hispanicus* (both 2n=52) were shown by Ainouche and Bayer (1999) to have genetic affinity, but were clearly distinct based on nucleotide changes. Both species are distributed in the Iberian Peninsula and separated by reproductive barriers; however, Swiecicki et al. (1999) claimed to have produced interspecific progeny from *L. luteus*×*L. hispanicus* subsp. *hispanicus.* Referred to as *'L.*×*hispanicoluteus',* it was similar to the two-parent species in chromosome morphology (Swiecicki et al., 1999; Naganowska and Ladon, 2000). Spontaneous autopolyploids are also known in *L. luteus* (2n=104) (Kazimierski, 1984). Kazimierski and Kazimierska (1975) showed that Israeli populations of *L. luteus* differed from western Mediterranean populations based on cytological and morphological evidence and claimed partial sterility of F_1 hybrids. They described the Israeli form as subspecies *orientalis* and noted that it had smaller seeds and was later flowering than the western Mediterranean types. Gladstones (1998), from observations of the different forms growing together, did not support their classification as a subspecies. There are also reports of wild ecotypes of *L. luteus* from Greece and Turkey that are probably closer morphologically and genetically to western Mediterranean cultivated bitter forms (Kazimierski and Kazimierska, 1975; Kurlovich, 1994; Kurlovich, 2002b). *Lupinus hispanicus* Boiss. and Reuter has been divided into subspecies by Gladstones (1974), who describes two fairly distinct forms existing in the Iberian Peninsula. Subspecies *hispanicus* was in accordance with the original type of Boissier and Reuter, which is similar to *L. luteus,* but flowers and seeds differentiate it reasonably clearly. Subspecies *bicolor* has been described as a variety of *L. hispanicus,* of

L. luteus, and even as species *L. rothmaleri* Klink. (Gladstones, 1984). *Lupinus hispanicus* ssp. *hispanicus* and ssp. *bicolor* did not form fertile hybrids with *L. luteus* but within and between *L. hispanicus*, there was a relatively high production of fertile F_1 progeny with intermediate characteristics (Gladstones, 1984). Other authors report the potential for some introgression between *L. luteus* and *L. hispanicus* (Lamberts, 1958; Kazimierski and Kazimierska, 1970).

The albus section comprises *L. albus* (2n=50) with its subgroups var. *albus* and wild forms var *graecus*, and the previously described species *L. graecus*, *L. juvoslavicus*, and *L. vavilovi* are considered to be blue-flowered, dark-seeded wild forms synonymous with var. graecus (Gladstones, 1974; 1984). A numerical taxonomic study by Nowacki et al. (1988), using 19 morphological characters on 19 genotypes, recommended the separation into separate species; however, earlier crossing studies by Kazimierski (1964a) showed no genetic barriers between wild forms and var. *albus*. Recent RAPD-based evidence also tends to support the proposal of botanical varieties rather than separate species. Spontaneous autopolyploids of 2n=100 were reported by Kazimierski (1984), but this phenomenon is probably rare. A strict consensus tree clade described by Ainouche (1999) grouped *L. albus* (2n=50) with *L. micranthus* (2n=52) and, together with isozyme data (Wolko and Weeden, 1990b), suggest the close affinity of *L. micranthus* with the smooth-seeded Old World species. Clements et al. (1993) noted that *L. albus* and *L. micranthus* were similar among the Old World species in their prolific production of cluster (proteoid) root structures. Other evidence places *L. micranthus* somewhere between the smooth-seeded and rough-seeded Old World lupins (Williams et al., 1983; Cristofolini, 1989; Wolko and Weeden, 1990a). Further, morphological parameters of fruits and seeds together with peroxidase enzyme data suggested *L. micranthus* was closer to *L. angustifolius* than to *L. albus* var. *graecus* (Drossos et al., 1996). The evidence however, generally supports that *L. micranthus* and *L. albus* are genetically closer to the rough-seeded group than are *L. luteus*, *L. hispanicus*, and *L. angustifolius*. Additionally, *L. albus* (and therefore *L. micranthus*) appears to have an intermediate position between the Old World and western New World species (Kass and Wink, 1997b; Ainouche and Bayer, 1999; Merino et al., 2000; Talhinhas et al., 2004).

Lupinus angustifolius has had a range of synonyms associated with it in the past, including *L. varius* L., *L. linifolius* Roth., *L. reticulatus* Desvaux, and *L. philistaeus* Boiss., all of which referred to the shorter, finer-leafed and smaller-seeded wild types. These were in contrast to the larger-leafed, larger-seeded types referred to as *L. angustifolius*, which probably resulted from some deliberate selection over time. *Lupinus opsianthus* Atab. et Maiss. referred to a small-seeded wild ecotype from Portugal (Atabiekova and Maisurjan, 1968). Some past subdivisions of the species include *L. angustifolius* van *basalticus*, a form with dark flowers, broad leaves, and pods, distributed in Israel on basalt soils that contrasted to "typical" var. *L. angustifolius*, growing on coastal sandy soils (Plitmann, 1966). A pink-flowering form, referred to as var. *basalticus* from Israel, was noted by Pazy et al. (1977), but this, along with the other var. *basalticus* types, was assessed by Gladstones (1984) to be very similar in morphology to cultivars of northeastern Europe that may have been introduced to Israel. Hanelt (1960) suggested that northern European cultivars were of Iberian pedigrees. Little or no genetic incompatibility is reported between the wild and primitive cultivars (Kazimierski, 1964b). Gladstones (1984) only mentions some minor fertility reduction in some crosses

between modern cultivars and wild types of eastern Mediterranean origin. Given the large spectrum of variation across the naturally occurring populations of *L. angustifolius*, the division between typical wild versus larger-seeded types is difficult. Recently Kurlovich and Stankevich (2002) put forward a number of subdivisions especially for *L. angustifolius*, *L. albus*, and *L. luteus*, including subspecific and botanical variety groupings. Further, a large number of agroecotypes were described (Kurlovich, 2002b). Acceptance of these taxonomically will no doubt be followed up, and the various proposed subtypes should be useful as the basis for new intraspecific taxonomic and phylogenetic research based both on morphological characters and at the DNA level (e.g., Ainouche and Bayer, 1999). The information could also be useful for the development of core collections by genebanks and selection of representative types to include in breeders crossing programs looking to broaden their genetic base.

9.3.1.2 New World Lupin Species

A review of the South American lupins was the Serie Species Lupinorum published by C.P. Smith from 1938 to 1945 and then by Macbride (1943). Several hundred species were described, based only on superficial characters, and many of the taxa were considered later to be synonyms (Planchuelo-Ravelo, 1984). Maisurjan and Atabiekova (1974) distinguished 12 sections among the New World lupins, and since then the studies conducted by D.B.Dunn and A.M.Planchuelo have attempted to clarify the complexities of the numerous and diverse New World *Lupinus* species using standard taxonomic descriptions, photographs of herbarium specimens, and flower dissections of type and nontype specimens. The phenotypic plasticity, presence of both annuals and perennial species in North and South America, ability to adapt to diverse environments, and considerable outcrossing have made taxonomic delimitation very difficult, and many published taxa are often no more than ecotypes (Planchuelo, 1994). Dunn (1984) demonstrated the heterogeneity of populations, noting that the *L. mexicanus-L. exaltatus* complex in Mexico contains both annual and perennial species, which are morphologically indistinguishable and interfertile. Planchuelo (1978) reviewed taxa from Argentina and reduced from 85 to 29 the number of species for that region. Further taxonomic studies of the species of that region, e.g., the *L. gibertianus-L. linearis* complex, are ongoing (Planchuelo and Fuentes, 2001). Interestingly, Dunn (1984) and Planchuelo-Ravelo (1991) identified the *L. gibertianus* complex in Uraguay as potentially related to *L. angustifolius* in the Old World. Many of the North American and Mexican species have been reviewed by Dunn (1979, 1984), and Barneby (1989) reviewed the Intermountain western U.S. species to assess synonyms and reduce the taxa from 200 to 21 species. Mexican and Central American lupin taxonomic research is still required to review the large number of published names for the region, many of which may prove to be synonyms (Planchuelo, 1999).

The predominant chromosome number for the New World lupins is 2n=48. Some species or individuals for which counts are recorded have 2n=96 (Dunn, 1984; Gillet et al., 2000). These species are toward the northern and high altitude limits of distribution and Dunn (1965) reported hybrids between Alaskan species with 2n=48 and 96. Turner (1957, 1994) reported 2n=36 for the majority of closely related species in northeastern Mexico, with an exception of 2n=24 for *L. caballoanus*. Lupin species morphologically

similar to those distributed in Mexico and Texas are found in South America, such as the *L. gibertianus-L. linearis* complex. Recent studies confirmed the existence of a species with 2n=36 chromosomes, the South American *L. albescens* Hook. Et Arn., *L. paraguariensis* Chodat et Hassl., and *L. multiflorus* Desr., with a species introduced to that region, *L. arboreus*, having 2n=48 (Perisse et al., 2000). Dunn (1984) also refers to a count of 2n=24 in a specimen of *L. aridus* Dougl. A range of southeastern South American species has predominantly 2n=36 with exceptions of 2n=32 and 34 (Maciel and Schifino-Wittmann, 2002). Subdivision of the New World lupins on the basis of morphological and genetic relationships and distribution based on studies by Planchuelo-Ravelo (1984) and Planchuelo (1994) help to simplify this large number of species (see discussion of centers of diversity and distribution). Several studies lend support to the genetic separation of the western New World (western North and South America), the eastern New World (including east-central parts of South America and the southeastern U.S.), and the Old World species (Kass and Wink, 1997b; Ainouche and Bayer, 1999; Maciel and Schifino-Wittmann, 2002). The western New World species are possibly closer to the Old World species than they are to the eastern New World species (Ainouche and Bayer, 1999). The studies also indicate a relatedness (although wide) of the two species *L. albus* and *L. micranthus* to the western New World species (Kass and Wink, 1997b; Ainouche and Bayer, 1999). The Central American species such as *L. mexicanus* and *L. elegans* (Mexican), and South American *L. mutabilis* (Andean) and *L. microcarpus* (N and S America), are grouped together with the western North American species (Ainouche and Bayer, 1999). The close relationship between *L. mutabilis* and its possible relatives (e.g., *L. bogotensis* Benth., *L. cruckshanskii, L. condensiflorus, L. paniculatus, L. malacotrichus*) to the North American species is also supported in serological (Cristofolini, 1989), isozyme (Wolko and Weeden, 1990b), and DNA studies (Kass and Wink, 1997b). The implications of these relationships with respect to genepools is further covered under section 9.5.2.

9.3.2 Origins

The origin of *Lupinus* has been contentious, with four different centers of origin having been proposed for the genus: Mediterranean-African region, North America, South America, and East Asia (Cristofolini, 1989). Lupins are assumed to have originated from the Sophoreae, the primitive tribe of the subfamily Papilionoideae, which in turn is thought to have evolved from the legume subfamily Caesalpinioideae in the lower Tertiary period (Polhill et al., 1981). The major theories of the evolution of lupins to their present distribution fall into two evolutionary pathways summarized by Gladstones (1998) as the Northern Hemisphere Genisteae pathway and the Southern Hemisphere pathway. The Northern Hemisphere Genisteae pathway proposes that the Genisteae tribe evolved from the Thermopsideae tribe distributed in the Northern Hemisphere, which in turn arose from the African Sophoreae. Plitmann (1981) proposed that *Lupinus* originated in North America from an originally Old World Thermopsideae ancestor, which spread to the Mediterranean and South America through long-distance transport via birds and land migration. The evidence of the genetic dissimilarity between Old and New World species and between the western and eastern American species, together with the closer genetic affinity of the eastern American species with the Old World group (Kass and Wink,

1997b; Zimniak-Przybylska and Przybylska, 1997; Ainouche and Bayer, 1999; Maciel and Schifino-Wittmann, 2002), tends not to support a single North American origin. Kass and Wink (1997b) provided support for an origin in the Old World and an independent colonization of the eastern New World, separate to the western New World species. This did not support the hypothesis that the Old and New World species were separated by continental drift, but species dispersion by long-distance transport across oceans from Europe or Africa westward to America. Cristofolini (1989) put forward the Asian Sophoreae and other Genisteae as the progenitor and hypothesized migration eastward and westward from there to the New and Old Worlds, respectively, followed by further independent evolution. Dunn (1984) and Gross (1988) argued that *Lupinus* originated in South America from *Crotalaria* and evolved probably following the Atlantic disjunction. This is based on the similarity between the simpleleafed lupins of eastern South America and *Crotalaria,* which is found primarily in tropical or subtropical southern Africa and whose tribe (Crotalarieae) has been regarded as a sister group to the Genisteae (Ainouche and Bayer, 1999).

Gladstones (1998) argued for a Northern Hemisphere Genisteae origin with modifications. This was based on early work and later DNA studies (Kass and Wink, 1994, 1997 a, b; Ainouche and Bayer, 1999), which revealed that *Lupinus* was not derived directly from Sophoreae, Thermopsideae, or Crotalarieae but, like those, a branch from a progressively evolutionary stream with Crotalarieae and Genisteae as recently diverged, related tribes. The Gladstones (1998) interpretation of the evolution of the geographical groupings can be summarized starting firstly from a subtropical African origin, followed by a northward spread on land, then an eastward movement toward the American continent. The progression began with the branching off of the simple-leafed species of warm-temperate eastern South America some 12 to 14 million years ago; secondly by the rough-seeded group of North Africa and Mediterranean regions; and then the smooth-seeded Old World species. The western American species, being the most highly evolved group, were the end point of this evolutionary stream perhaps 3 to 4 million years ago, with further movement into the Andes of western South America. The first two stages are supported also by the cytological studies of Maciel and Schifino-Wittmann (2002). The similarities of habitat and morphology of the simpleleafed lupins to Crotalaria are explained by a branching that occurred before tribal differentiation was complete. The hypothesis explains the similarity of *L. mutabilis* to some west coast North American species. Kazimierski and Novacki (1961) suggested that *L. mutabilis* arose from hybridization between the two North American species *L. douglasii* L. and *L. ornatus* L. The present northerly and high altitude species in America have probably immigrated and evolved *in situ* since the end of the last ice age, approximately 12,000 years ago (Gladstones, 1998). The chromosome numbers of 2n=48 and 96 in some more northerly or high-altitude limits of distribution are consistent with the theory of greatest polyploidy following active recent evolution and colonization (Stebbins, 1985). Cristofolini (1989) proposed the origin could have been more north in the Sino-Himalayan region of Asia. The Old World rough-seeded lupin, with its range of lower chromosome numbers, Gladstones (1998) proposes to be relicts of the early northward evolution from the subtropics, and the recent increase in number of chromosomes in some species of this group was due to individual chromosome duplications or subdivisions as a result of evolution in changeable environments. The smooth-seeded Old

World species have higher chromosome numbers ranging from 2n=40 in *L. angustifolius* to 2n=52 in *L. luteus* and *L. hispanicus*, with the latter group possibly still evolving in the cool northern limit of the genus in Europe. Gladstones (1998) proposes that the place of origin of the smooth-seeded Mediterranean and western American lupins is likely to be Turkey, Syria, and surrounding areas. General trends consistent with the hypothesized evolutionary pathway were noted by Gladstones (1974, 1998) for Old and New World lupins: a pattern of lower chromosome numbers in the warmer regions progressing to higher chromosome numbers in cooler, higher altitude or latitudes; and species with highest chromosome numbers and current or recent active evolution appear to have the highest incidence of natural outcrossing.

9.3.3 Centers of Diversity and Distribution

The centers of diversity of *Lupinus* were considered by Hondelmann (1984, citing von Sengbusch) to be (1) Central America, (2) the Andean region of South America, and (3) the Mediterranean and northern and eastern African region. Based on recent molecular evolution and other studies that revealed genetic similarities, the New World centers of diversity can instead be divided into (1) North and Central America and Andean South America, and (2) Atlantic South America (Planchuelo, 1994; Zimniak-Przybylska and Przybylska, 1997; Ainouche and Bayer, 1999; Maciel and Schifino-Wittmann, 2002). The distribution maps of the Mediterranean species can be found in Gladstones (1974, 1998).

9.3.3.1 Lupinus albus

The semidomesticated, large-seeded forms of *L. albus* (var. *albus*) have long been cultivated around the Mediterranean and in the Nile Valley, but the primary center of diversity is implied from the reported distribution of the wild *L. albus* var. *graecus*, which includes the southern Balkans (Greece, including Crete, Albania, and the former Yugoslavia) and possibly into northeastern Greece, southern Italy, and western Turkey (Perrino et al., 1984; Clements and Cowling, 1990; Gladstones, 1998; Cowling, 2001). The natural distribution of the derived cultivated landraces, *L. albus* var. *albus* is circum northern Mediterranean basin, the mid-Atlantic islands (Azores), the Canary Islands, north Africa (Morocco, Algeria, Tunisia) and the Nile Valley, Kenya, and Ethiopia (Buirchell, 1992; Gladstones, 1984, 1998; Neves-Martins, 1994). Generally, *L. albus* is distributed on mildly acid or neutral soils of light to medium texture. Some occurrences on alkaline soils are reported in regions such as Egypt, Corsica, Malta, Sicily (Palermo), and the Atlantic Islands (e.g., Verde Islands).

9.3.3.2 Lupinus luteus

Lupinus luteus is more confined to acid soils, and natural populations are much less widespread than *L. albus* or *L. angustifolius*, and wild forms are rare. The suggested place of origin is the Iberian Peninsula (Gladstones, 1974) and the main current distribution center is in the western parts of this region. Smaller-seeded, possibly wild types occur in

the high rainfall areas of Portugal and northwest Spain, with some reports of occurrences in southern Spain, southern Italy (Calabria, Sicily), Greece, Turkey, coastal Morocco, and Israel and Lebanon (Kazimierski and Kazimierska, 1975; Gladstones, 1974, 1984; Cowling, 2001; Kurlovich, 2002b). Occurrences in the eastern Mediterranean support the hypothesis of a temperate western Asian origin of the smooth-seeded lupin species (Gladstones, 1998). *Lupinus luteus* landraces are scattered along with the wild forms and as escapes from cultivation or use as an ornamental. Generally, *L. luteus* is distributed on sandy, acid soils.

9.3.3.3 Lupinus angustifolius

The Aegean region is the possible center of diversity of *L. angustifolius*, with small-seeded, finer-leafed genotypes occurring particularly at higher elevations in northern Greece and some islands (Clements and Cowling, 1994; Cowling, 2001). Gladstones (1998) suggests possible wild types distributed in North Africa and Iberia. A continuous range from small-seeded to larger-seeded types are found, with the latter types often associated with present or possible past agricultural activity, consistent with the idea of selection by early farmers. *Lupinus angustifolius* natural populations are the more widespread than the other Old World lupin species. Generally, *L. angustifolius* is found on well-drained, noncalcareous, light to medium textured soils.

9.3.3.4 Other Smooth-Seeded Old World Species

Lupinus hispanicus, which is closely related to *L. luteus* but whose distribution extends to higher altitudes, is found mainly in Spain and Portugal (Gladstones, 1974). *Lupinus hispanicus* ssp. *hispanicus* is found at moderate altitudes in southern and central Spain, and *L. hispanicus* ssp. *bicolor* at higher altitudes up to 1,500 m in northwestern Spain and northern Portugal, sometimes on poorly drained soils. There have been some reports of occurrences in Turkey and Northern Greece (Gladstones, 1998; Clements and Cowling, 1990; Cowling, 2001). *Lupinus hispanicus* is generally distributed on sands to sandy loams that are acidic to very acidic (Gladstones, 1974). *Lupinus micranthus* is relatively rare but is the second most widespread Old World species after *L. angustifolius* (Gladstones, 1974). Its distribution is around the perimeter of the Mediterranean basin, and this implies its translocation through human activity over time. It is found on mildly acid to alkaline soils, frequently on sandy loams, but also on coarse sands and heavier and more calcareous soils.

9.3.3.5 Rough-Seeded Lupins

The seven recognized rough-seeded lupin species mostly have a very restricted natural distribution, and their habitats generally show little overlap. These species still exist as wild plants, with evidence of some human selection for larger seeded or ornamental types in *L. pilosus*, *L. digitatus*, *L. palaestinus*, and *L. atlanticus* (Gladstones, 1974, 1998; Plitmann, 1981). Distributions of the rough-seeded species in northern Africa, eastern Mediterranean, and southern Portugal and Spain are shown in Figure 9.3. Their habitats

range from desert wadis to tropical highlands; from high in the mountains to coastal plains; from acid soils to highly calcareous soils. They encompass most environments and soil types, although predominantly from neutral to alkaline soils. Some limited overlap occurs between *L. pilosus* and *L. palaestinus* in Israel. Some Israeli forms of *L. pilosus* appear closer in form to *L. palaestinus;* this suggests the distribution of possible hybrids where the two species grow together (Clements et al., 1996; Gladstones, 1998). Little penetration of the rough-seeded lupins into the northern Mediterranean has occurred with their specialized adaptation to semidesert and warm Mediterranean conditions. *Lupinus pilosus* has a diverse distribution from the mountainous regions of Crete and Turkey to the Greek Islands to the coastal areas of Israel and Syria. It grows on a wide range of soil types, having been collected from coastal sandy soils to loamy clays with limestone present. It has been mostly reported to prefer sandy soils of neutral to alkaline pH. The species seems well adapted to a number of climatic environments, and there is considerable variation within this species. *Lupinus palaestinus* is a low-growing plant, with much basal branching and long inflorescences. Its natural range extends from the central and southern plains of Sharon and Philistia, down the coast of Israel. There is also a pocket of the species found on the Sinai Peninsula in the Jebel El Tih. In the Sinai, this species probably takes advantage of moist conditions in the bottom of wadis. *Lupinus atlanticus* was named by Gladstones in 1974 following a collection mission to Morocco in 1972. This distinctive species is restricted to the Atlas and Anti Atlas Mountains of Morocco at altitudes less than 1700 m but greater than 460 m. The northern extent of its habitat is around Beni Mellal, and in the Atlas Mountains it has only been found on the western slopes. Some plants have been collected in the deeper valleys of the Atlas Mountains, but the main area seems to be the foothills of the western slopes. In the anti-Atlas

Mountains it is found in the valleys of the central mountains around Tanalt, in the valleys around Tafraoute, and on the Kerdous Plateau. The southern boundary is unknown, but it is restricted to above 250 mm rainfall.

The geographically isolated *L. princei* is recorded in the highlands of Kenya, Tanzania, and southern Ethiopia at elevations between 1700 and 3000 m. The species is thought to have been isolated in that region after temperature increases during interglacial periods (Gladstones, 1998). The extent of its current natural habitat in Ethiopia is unknown; however, there should be suitable habitat in regions of Ethiopia other than the southern highlands. *Lupinus digitatus* is known from a number of regions in the southern Sahara, mostly associated with mountainous areas. It has been found on the border of Chad and Libya, Niger and Algeria, in the Senegal Valley, the North West Sahara and in the Nile Valley. The only seed in international seed banks have come from a single collection from the Wadi Karit, east of Kom Ombo oasis in southeast Egypt. The species was noted as a common winter volunteer in cultivated fields in the Nile Valley but has disappeared as a consequence of the damming of the Nile at Aswan. This species is also likely to be more restricted than Figure 9.3 shows, due to desertification within most of its range. It is clearly a species that needs collecting. The extent and status of the distribution of *L. somaliensis* is unknown, but may extend from the region of its type specimen in the Golis Range of northern Somalia into Ethiopia. It is possible that *L. somaliensis* may be a variation of *L. princei*.

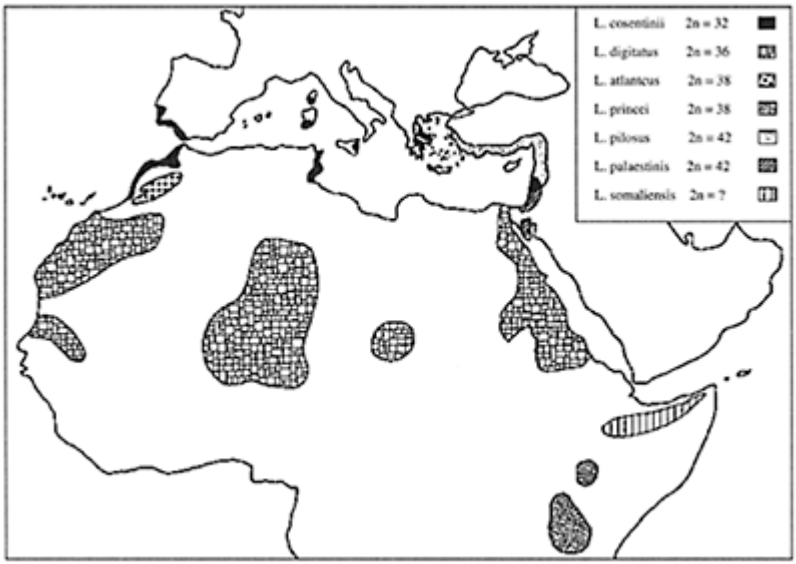

Figure 9.3 Natural distribution of the seven species are deduced from botanical collections published in the literature (Gladstones, 1974) and seed collection missions.

9.3.3.6 New World Lupins

Based on the available published reports and studies of the biodiversity and genetic interrelationships by Dunn and Planchuelo, the New World lupins have been subdivided into geographic regions (Dunn, 1984; Planchuelo-Ravelo, 1984; Planchuelo, 1994). Overall, they are distributed across a wide range of climates including alpine, temperate, and subtropical. The centers of diversity are considered to be within these regions and are primarily North and Central America, the Andean South American region, and the Atlantic South American region, which encompasses the majority of the primitive simple-leafed species. Planchuelo (1994) divides the North and Central American region into the Southeastern Subregion (SEN) and Mountain Range (from Alaska to Central America Subregion (MAC)). The SEN group consists of four simple-leafed species (thought to originate from Brazil) in coastal North Carolina to the Mississippi in the Gulf of Mexico. The MAC group of compound-leafed lupins extends from the Aleutian Island in Alaska, along and on both sides of the mountains and ranges in North America, to the Sierra Madre in Mexico and Central America. The group includes ornamental and forage species *L. polyphyllus,* and soil conservation species *L. arboreus* and *L. nootkatensis,* and the ornamental *L. mexicanus* (synonyms *L. hartwegii, L. ehrenbergii*). The South American region is divided into the Atlantic Subregion (ATL) and Andean Subregion

(AND). The ATL group consists of a large number of perennial simple and compound-leafed species and a small group of variable annual species. It includes the *L. gibertianus-L. linearis* complex, which is thought to have some genetic similarities to *L. angustifolius*. The group is distributed across eastern Brazil, Uruguay, Paraguay, and central and eastern Argentina. The AND group covers the geographic region from the mountain slopes on both sides of the Andes, from Venezuela through Colombia, Ecuador, Peru, Bolivia, Chile, and northwest Argentina, to the plains of Patagonia in the south. The species consist of perennial and annual compound-leafed species. The range in topography and microclimates create a large ecological diversity of plant types (Planchuelo, 1994). Within the AND group is *L. mutabilis*, the only crop species from the region. The natural distribution of *L. mutabilis* is difficult to ascertain, but reports of its cultivation are from as far north as Venezuela through Colombia, Ecuador, Peru, Bolivia to Chile and northern Argentina (Williams, 1979; Blanco, 1982; Planchuelo and Dunn, 1984; Mujica, 1994; Planchuelo, 1994; Office of Internal Affairs, 1989) (Figure 9.4).

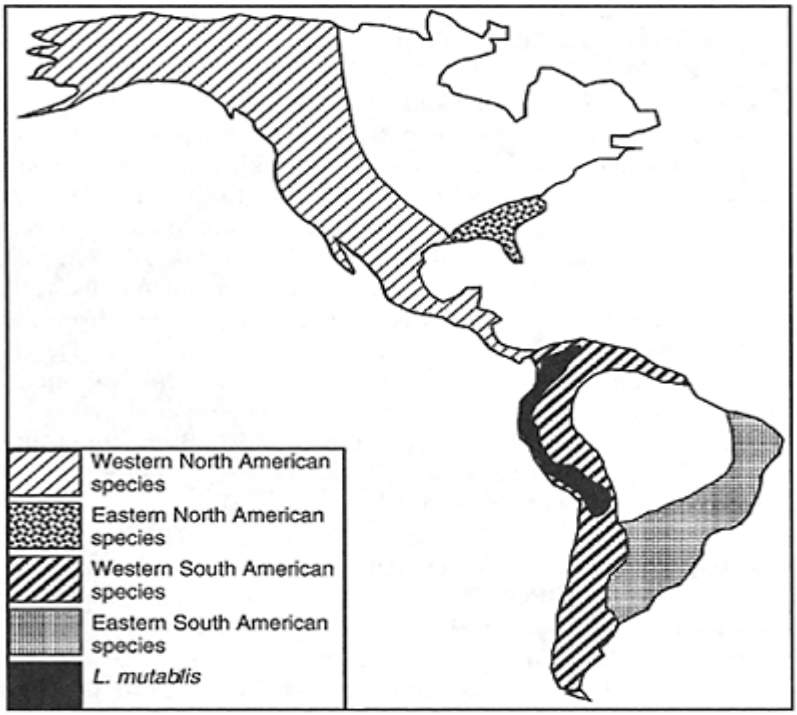

Figure 9.4 Approximate distribution regions of New World lupin species including *Lupinus mutabilis* (from Planchuelo-Ravelo, 1984; Mujica, 1994; Planchuelo, 1994; Various collection site data sources).

9.4 GERMPLASM RESOURCES

9.4.1 Germplasm Collections and Genebanks

Modern breeding of lupins is relatively recent, having only begun 70 years ago, and genetic resources will play a crucial role in their further improvement for agriculture. The importance of collection, conservation, documentation, and evaluation was recognized by early breeders in Germany, Holland, Poland, and Russia in the original domestication and development of the first cultivars. Crosses between domesticated types and wild or landrace types are still an important component of breeding programs for improving disease and pest resistance and tolerance to abiotic stresses (Cowling et al., 1998b). Human activities have greatly impacted lupin populations, especially in the Mediterranean and North African regions. This has both diminished the occurrence of certain wild lupins and increased the spread of other wild or semidomesticated types.

In 1926, N.I.Vavilov conducted a germplasm collection trip that included northern Africa, Cyprus, Crete, Sicily, Sardinia, Spain, Portugal, France, and Greece. Among the hundreds of species were germplasm of *Lupinus* (Kurlovich et al., 2000). The next expedition of importance was that of Klinkowski (Klinkowski and Hackbarth, 1941), which focused on *L. luteus* and *L. angustifolius* from Spain and Portugal. However, these materials were lost during the Second World War. Lamberts (1955) collected *L. luteus* after the war, but it was not until collections made by J.S. Gladstones that contributions to lupin genetic resources collection and evaluation gained momentum (Gladstones, 1973, 1974; Gladstones and Crosbie, 1979; Swiecicki, 1988a). During the 1980s, extensive collections of lupin germplasm were made, especially from the Iberian Peninsula and Greece, and evaluated by a number of scientists (De Haro et al., 1982; Mota et al., 1982; Mota, 1984; Jambrina and Crespo, 1984; Simpson and Neves Martins, 1984; Cowling, 1986; Simpson, 1986a, 1986b; Papineau and Huyghe, 1989). It was also during that decade that computerization and improved dissemination of information occurred through the International Plant Genetic Resources Institute (previously IBPGR) (IBPGR, 1986, 1989). This included the publication of lupin descriptors (IBPGR, 1981). Additionally, the establishment of the International Lupin Association and the ensuing International Lupin Conferences (López Bellido, 1991) undoubtedly facilitated the exchange of knowledge and germplasm. Summaries of lupin genetic resources have since been published in international legume conferences (Swiecicki, 1988a; Swiecicki et al., 1999), and books (Buirchell and Cowling, 1998; Cowling et al., 1998a; Cowling, 2001). The Database of European *Lupinus* Collections was initiated in 1995, and details of lupin accession holdings in Europe were published with other grain legumes (IBPGR, 1989; IPGRI, 1995). More recent collections and evaluation studies of lupin germplasm collections include the following: *L. angustifolius* of the Aegean region (Clements and Cowling, 1994); *L. albus* from the Azores Islands (Huyghe et al., 1990), from Portugal (Neves-Martins, 1994), from Spain (De Haro et al., 1982), and from Egypt (Christiansen et al., 1999, 2000); *L. albus, L. angustifolius,* and *L. luteus* from the genebank at the Instituto Superior de Agronomia, Portugal (Pereira et al., 2000); *L. pilosus* (Clements et al., 1996); *L. atlanticus* from Morocco (Buirchell, 1992; Buirchell, 1996; Cowling et al., 1998a); *L. albus* from Ethiopia (Francis et al., 1997); *L. angustifolius, L. albus, L. luteus, L. cosentinii,* and *L. atlanticus* from Morocco (Alami et al., 2004).

Collections of *L. mutabilis* were first made in Peru in the 1970s through the University of Cusco, with further missions involving Universidad del Centro in Huancayo and Universidad de Puno, often with support from the IBPGR. The National Lupin Germplasm Collection of Peru was initiated at the Santa Ana research station in Huancayo/INIA, with support from the German Agency for Technical Cooperation (GTZ) (Blanco, 1974; Herquinio and Román, 1974; Velasco, 1986; Binsack, 1991; Cowling et al., 1998a). Collection and evaluation in Bolivia was conducted by the Pairumani Research Station and in Ecuador by the Instituto Nacional de Investigación Agropecuarias (INIAP). Germplasm evaluation studies or summaries of *L. mutabilis* germplasm include Blanco (1982), Tapia and Vargas (1982), Ortega and Rodriguez (1984), Neves-Martins (1994), Neves-Martins and da Silva (1994), and Planchuelo (1999).

Current total holdings of *Lupinus* germplasm accessions around the world, according to the IPGRI Directory of Germplasm Collections Database (IPGRI, 2004) are estimated to be 40,000. Collections of greater than 40 *Lupinus* accessions are listed in Table 9.2. Collections greater than 1,000 accessions of Old World lupins are held by: Australian Lupin Collection (ALC), Australia (the Australian Temperate Field Crops Collection, Horsham holds duplicate accessions from the ALC); Vavilov Institute, Russia; Junta de Extremadura. Servicio de Inv. y Desarrollo Tecnológico Finca la Orden, Spain (particularly Spanish *L. albus, L. angustifolius, L. luteus*, and *L. hispanicus*); Centro de Recursos Fitogeneticos, INIA, Spain; Station d' Amélioration des Plantes Fourragères, INRA, France; the Genebank, Inst. for Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany; the University of Reading, England; the Plant Breeding Station, Wiatrowo, Poland. The Australian Lupin Collection and Vavilov Institute collections appear to be the only ones in which Old World lupin species are represented, except for the possibly extinct *L. somaliensis*. The ALC has detailed passport, collection site, preliminary evaluation, and inventory data and includes a large collection of the rough-seeded species, which includes 217 *L. cosentinii,* 163 *L. pilosus,* and 89 *L. atlanticus.* Lists of accessions with passport and evaluation data for that collection have been made available via the Internet (Cowling et al., 1998a; Smith, 2004). The Vavilov Institute preserves 28 species of *Lupinus* and has considerabe evaluation data available in Russian, although much of it is not published (Vishnyakova and Lubov, 2004). Substantial collections of *L. hispanicus* (having the same chromosome number as *L. luteus*) are held in Spain, such as in the Departamento de Pastos y Forrajes, Salamanca.

Significant collections of New World species, notably *L. mutabilis,* are held in South American institutions such as Centro Regional de Investigacion en Biodiverisdad Andina (UNSAAC), Estacion Experimental Agropecuaria Santa Ana, INIA, and Universidad Nacional San Antonio Abad del Cusco (UNSAAC/CICA), with all three located in Peru and the latter institute holding *L. mutabilis* originating from Argentina, Bolivia, Columbia, Ecuador, and Peru. Some *L. mutabilis* collections are held outside South America, for example by the Federal Centre for Breeding Research on Cultivated Plants (BAZ), Germany, the Granja-Escuela de Capacitación y Experimentación Agropecuaria, Huelva, Spain, and Station d'Amélioration des Plantes Fourragères, INRA, France. The Western Regional Plant Introduction Station, USDA-ARS, Pullman, holds a diverse collection of lupins including more than 50 New World species, and relatively large numbers of American species are held in Gatersleben, Germany, and by the Institute for

Agrobotany, Hungary. The Vavilov Institute holds a significant collection of *L. polyphyllus*, *L. mutabilis*, and other New World species.

Overall, approximately half (20,000) of the world's genebank-preserved *Lupinus* accessions are of the three major domesticated, smooth-seeded species, *L. albus*, *L. angustifolius*, and *L. luteus*, and one quarter are *L. mutabilis*. There are only approximately 1,000 rough-seeded accessions, and, of these, there are only very small holdings of *L. digitatus*, *L. palaestinus*, and *L. princei*. The lesser-known, Old World, smooth-seeded species *L. micranthus* is poorly represented, the largest holdings (43 accessions) available from the ALC. The *L. albus* wild form var. *graecus* is also not well preserved compared with landrace and domesticated accessions of the species (var. *albus*). Although a relatively large number of accessions are conserved for *L. mutabilis*, information on their passport, collection site, and preliminary evaluation requires further development. Free access to these already quite substantial collections is another issue that faces breeders interested in this crop (Swiecicki et al., 2000). With the range of geography and microclimates of sites in the region of diversity of *L. mutabilis* and related species, in addition to the start of genetic erosion (Blanco, 1986; Planchuelo, 2000), it is of high priority to document existing germplasm collections to determine duplication and deficiencies.

Table 9.2 World *Lupinus* Germplasm Collections (of >40 Accessions)

Institute Country	Institution	Total Lupin Accessions	*L. albus*	*L. angustifolius*	*L. luteus*	*L. mutabilis*	*Other New world*	*Rough-seeded*	*Comments*
Australia	Australian Lupin Collection*	3639	873	1793	224	104	31	488	All Old World sp. represented (except *L. somaliensis*)
	Australian Temperate Field Crops Collection	3177	794	1557	202	72	56	372	
	CSIRO Division of Plant Industry	86	21	26	26	13			
Bulgaria	Institut de ressources phytogénétiques 'K. Malkov'	68							
Bolivia	Centro de Investigaciones Fitoecogenéticas	114				114			

	de Pairumani								
Canada	Saskatoon Research Centre	214	42	34	58	2	41		
Chile	Campex	183	70	4	4	100	4		
	Inst de Inv. Agropecuarias	1259	12						
Czechoslovakia	AGRITEC, Research, Breeding and Services Ltd.	74	37	11	26	1			
Germany	Federal Centre for Breeding Research on Cultivated Plants (BAZ)	1873	115	373	169	998	50	6	
	Genebank, Inst. for Plant Genetics and Crop Plant Research (IPK)	1674	190	465	792	25	63	17	
	State Plant Breeding Institute, University of Hohenheim	200							
Ecuador	Estacion Experimental Santa Catalina, DENAREF, INIAP	530							Large proportion from ECU, PER, BOL
	Instituto de Ciencias Naturales Universidad Central del Ecuador	130				130			
Egypt	Field Crops Institute Agricultural Research Centre (ARC)	88							
Spain	Centro de Recursos Fitogeneticos, INIA	1554	567	537	240	20	13	177	*L. hispanicus*

	Compania Espanola de Cultivos Oleaginosos, S.A. (CECOSA)	260							
	Departamento de Pastes y Forrajes	749	6	410	80			253	*L. hispanicus*
	Granja-Escuela de Capacitación y Experimentation Agropecuaria	436	15	6	15	400			
	Junta de Extremadura. Servicio de Inv. y Desarrollo Tecnológico Finca la Orden	1804	694	553	353			204	Especially Spanish *L. albus, L. angustifolius, L. luteus; L. hispanicus*
	Ramon Batlle Vernis, S.A.	254	112			142			
France	Station d'Amélioration des Plantes Fourragères, INRA	2046	1398		148	332			
Great Britain	Agric. Botany, Plant Sci. Lab., School of Plant Sc., Univ. Reading	1300	1100			200			
Greece	Greek Genebank, Agric. Res. Center of Makedonia and Thraki, NAGREF	87	9	70	1			1	
Hungary	Institute for Agrobotany	451							
Israel	Dept. of Botany, Institute of Life Science, Hebrew Univ. of Jerusalem	47		10	4		30	15	*L. palaestinus* accessions
Italy	CNR—Istituto di Genetica Vegetale	100							
	Dip. di Agronomia & Genetica Ve.g., Universita degli Studi di Napoli	55	48	3		22			
Kenya	Kenya Agricultural Research Institute	74	2	5	1	30			
	National Genebank of Kenya	101	20	9	6	28	5		

Lithuania	Voke Branch of the Lithuanian Institute of Agriculture	120						
Netherlands	Centre for Genetic Resources, The Netherlands (CGN)	69	13		56			
Peru	Centro Regional de Investigacion en Biodiverisdad Andina (UNSAAC)	1940		60	50	1800		*L. mutabilis* landraces from BOL, PER

Table 9.3 World *Lupinus* Germplasm Collections (of >40 Accessions) (Continued)

Institute Country	Institution	Total Lupin Accessions	*L. albus*	*L. angustifolius*	*L. luteus*	*L. mutabilis*	*Other New World*	*Rough-seeded*	*Comments*
	Estacion Experimental Agraria Baños del Inca, INIA	347				347			
	Estación Experimental Agropecuaria La Molina, INIA	50				50			
	Estacion Experimental Agropecuaria Santa Ana, INIA	1725				1725			*L. mutabilis* majority from PER
	Estación Experimental Illpa-Puno, INIA	138							
	Universidad Nacional Agraria La Molina	300				300			*L. mutabilis* from PER
	Universidad Nacional del Altiplano	319				319			
	Universidad Nacional San Antonio Abad del Cusco (UNSAAC/CICA)	1800				1800			*L. mutabilis* from ARG, BOL, COL,

										ECU, PER
Poland	Botanical Garden of the Polish Academy of Sciences	150								
	Plant Breeding and Acclimatization Institute (IHAR)	509								
	Plant Breeding Station Wiatrowo	1049	242	198	421		14	22	29	
Portugal	Banco de Germoplasma Genetica, Estacao Agronomica Nacional	855	270	240	210				11	Primarily Old World species
	Banco Português de Germoplasma Vegetal (BPGV)	81	69		12					Accessions from PRT
	Dept. de Botanica e Eng. Biologica Instituto Superior de Agronomia	661	377	37	78		149	5	15	
	Seccao de Forragens, Dept. Genet. Melh., Estacao Agronomica Nacional	58	20	12	26					
	Seccao de Genetica Estacao Agronomica Nacional	288		288						
Romania	Agricultural Research Station Livada-Satu Mare	100	100							
	Suceava Genebank	71	59	5			3 1 3			
Russian Federation	N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry**	3200	541	968	906	132	288	54	161 *L. polyphyllus*	
Slovakia	Research Institute of Plant Production	46	20	9	17					

	Piestany								
Ukraine	Institute of Agriculture, Chabany	910							
U.S.	Gold-Smith Seed Co., Inc.		160	50		200	25		
	Western Regional Plant Introduction Station, USDA-ARS	1081	321	184	79	79	303	18	More than 50 different New World species
South Africa	National Dept. of Agriculture	44	6	34	4				
	Plant Genetic Resources Unit, Agricultural Research Council	43	14	6	5	12	3		
Totals		**38581**	**8337**	**7957**	**4218**	**9641**	**899**	**1054**	

Source: IPGRI Directory of Germplasm Collections (IPGRI 2004, with permission); *Smith (2004), **Vishnyakova and Lubov (2004).

9.4.2 Genetic Diversity of the Smooth-Seeded Mediterranean Crop Species

The smooth-seeded Old World lupins have been found to be a more widely separated group of species than the rough-seeded group. However, when comparing the within-species variation among this group, recent molecular studies using AFLP, ISSR, and RAPD markers have revealed that *L. albus* and *L. mutabilis* each showed relatively narrow intraspecific genetic diversity compared to *L. angustifolius*, with *L. luteus* showing an intermediate level (Talhinhas et al., 2004). These results may reflect the major influence of human selection over centuries on species such as *L. albus* and *L. mutabilis*, compared to the more recently developed *L. angustifolius*. Landraces of *L. albus* were likely to have been selected and taken around the Mediterranean and Northern Africa under different empires over the past few millennia. Wild populations of *L. albus*, *L. mutabilis*, and *L. luteus* have apparently become scarce in this process of selection. From a breeding point of view, lupins are a relatively young crop and exploitation of existing genetic variation is only in its early stages. The following discussion is intended as a guide to the groups of germplasm that may provide different genetic variation for use in crop improvement programs.

9.4.2.1 Lupinus albus

Lupinus albus genetic diversity consists of the wild var. *graecus* types (see Centers of Diversity section), landraces (*L. albus* var. *albus*), and intermediate plant types, which occur wherever cultivated landrace and wild populations overlap such as in southern

Greece and Crete (Cowling, 1986; Simpson, 1986b). *Lupinus albus* var. *graecus* differs from var. *albus* in that it has dark, violetblue flowers, short stems, smaller, dark-brown impermeable seeds, and shattering pods. *Lupinus albus* var. *albus* has variably bluish-white flowers, white seeds, and nonshattering pods.

9.4.2.1.1 Iberian Peninsula, Balkans, Turkey, Egypt

Given the continuous range of morphological types, Simpson (1996a, 1996b), using multivariate statistical methods, usefully characterized four geographical races of *L. albus* in the Mediterranean region. These were: (1) the Iberian race, which was subdivided into two groups, one with large leaves, pods, and seeds from the Azores Islands, Catalonia (eastern Spain), Cadiz (southern Spain), and central Portugal, and the other early flowering with small leaves, pods, and seeds from Palencia and Leon (central-north Spain); (2) the Nile Valley race, with smaller and fewer main stem leaves (reduced vegetative development) and including shorter rosette growth stages and earlier flowering; (3) the distinct and homogeneous Turkish race (typically spring-sown), with very small leaves, pods, and seeds (200 mg/seed) and more lateral pods than main stem pods. This group included the earliest flowering types without vernalization requirement, and some genotypes had pink flowers (regarded as a botanical 'var. *subroseus*' *by* Kurlovich and Stankevich, 2002). Populations collected in coastal regions of Turkey were also earlier flowering but were more vigorous and had larger seeds (Huyghe, 1997); (4) the Balkan race (traditionally autumn-sown), which included wild var. *graecus* and cultivated genotypes with shorter stems and many main stem and lateral pods. These materials were found to have limited frost tolerance but good pod set (Huyghe, 1997). Huyghe et al. (1990) reported genetic variability among accessions collected on the Azores Islands for phenology and seed yield components with some potential for selection of *Pleiochaeta setosa* and anthracnose resistance. Approximately 200 Portuguese ecotypes were evaluated by Neves-Martins (1986, 1994), who described winter, spring, and intermediate types using a range of morphological characters. They noted a group of plants, which they named *'megalosperma'*, with large seeds and vegetative structures that was distributed near Leiria on the central coast. Seed sizes in *L. albus* include large and very large-seeded 'lupini' types (up to 1000 mg per seed), some of which can be found in Italy. Seeds with a pink hue are also found, particularly from the Iberian Peninsula and the Madeira (Portugal). A range of landraces from Portugal and Spain, many of which had local names, were evaluated by Awopetu (1988). They found that these landraces were all later flowering than control cultivars Kali and Kiev Mutant by a minimum of several days, but with a range from intermediate to late flowering, the latter suggesting these were selected as winter-type landraces, which often have a rosette stage during early development and respond to longer days to initiate flowering. The number of leaves on the primary inflorescence, being fewer in the cultivars, was highly correlated with time to flowering. Some high altitude, northern Iberian types, however, southeast with early-mid flowering types to the northwest with late flowering, small-seeded, typically winter-type growth habits—when grown in northern Europe. Italian

germplasm is considered to be very diverse, and frost tolerance can be found in material from the Apeninnines and Abruzzo. More vigorous but cold-sensitive types occur in coastal regions in the south of Italy (Hughye, 1997). Swiecicki (1988a) mentions *L. albus* with high diversity on the southern tip of Italy and from the valley of San Marteno, near Palermo, Sicily, and at the northern part of Corsica that are adapted to alkaline soils. Sources of resistance to fusarium wilt and anthracnose are reported to occur among wild-growing Iberian *L. albus* (Kurlovich, 2002b). The N.I.Vavilov Institute of Plant Industry preserves accessions of the traditionally cultivated forms of *L. albus*, known as 'Hanchcoly' from the former USSR state of Georgia, and these are considered to be of value for breeding (Kurlovich, 1996).

Egyptian landraces of *L. albus* have been regarded as relatively low yielding with medium to high alkaloid levels, and they are still of importance as a winter crop in the region. Minor collections of *L. albus* in Egypt by Australian scientists in the early 1990s highlighted genetic variation in traits such as early flowering and tolerance to alkaline soils. Material collected by Buirchell in 1993 in southern Egypt has provided some more vigorous material to that collected by Simpson. Egyptian germplasm collected in 1995–1996 as part of an Egyptian-Danish project consisted of landraces of heterogeneous flower color, indeterminate growth habit, with a range in vigor, duration of vegetative period, plant height, and yield-related traits (Christiansen et al., 1999, 2000). Some accessions were collected from highly calcareous soils with a pH above 8.5 and $CaCO_3$ content above 10%. Alkaloid levels were found to vary between 0.2 and 1.4%. Simpson (1996a, 1996b) indicated strong affinities of his Nile Valley race to southern Iberian populations. Evaluation of landraces from Egypt and Sudan under Western Australian growing conditions have shown that they generally have early vigor and early flowering, and this may reflect their selection for rapid spring growth under flood irrigation in the Nile Valley. Kurlovich (2002b) records some accessions from the Egyptian region as having high resistance to fusarium. Generally, Gladstones (1974) notes that types from the northern and western Mediterranean have large seeds and leaves with taller, stronger stems and are late flowering. Those from the southeast Mediterranean had smaller seeds and leaves, with earlier flowering and bluish-tinged flowers.

9.4.2.1.2 Ethiopia

Collections from Ethiopia were also assessed in Western Australia, and two groups were identified (Buirchell and Cowling, 1998). The first were normally large-seeded types resembling material from Sudan and Egypt. The other group had small seeds and leaves and showed some genetic incompatibility when crossed with *L. albus* from other stocks. Recent collections of cultivated landraces from the Lake Tana region at the source of the Blue Nile, Ethiopia, by Professor Clive Francis of CLIMA, The University of Western Australia (Francis et al., 1997) have provided small-seeded, fine-leafed types with an important source of resistance to anthracnose. Kurlovich (2002b) mentions that a few 'Abyssinian' *L. albus*, collected by N.I.Vavilov in 1927, have high protein content and some others from the Blue Nile as having drought and fusarium resistance.

Table 9.3 Variation for Plant and Seed Traits and Collection Site Data in *Lupinus albus, Lupinus luteus, Lupinus angustifolius* Wild and Landrace Accessions Held in the Australian Lupin Collection, Perth

Species		Height 9 Weeks (cm)	Days to Flowering	Seed Weight (mg)	Altitude (m)	Rain (mm)	Soll pH
L. albus	n	663	689	730	274	49	230
	min	3	59	112	1	350	5.0
	mean	26	91	329	792	539	7.9
	max	74	151	855	2600	1200	9.5
L. luteus	n	106	150	113	49	34	42
	min	3	90	53	10	300	5.5
	mean	14	116	96	158	656	6.3
	max	28	150	138	500	1100	8.5
L. angustifolius	n	1129	1218	1257	1084	691	857
Wild, landrace	min	3	75	30	1	200	4.2
	mean	16	104	109	381	621	6.7
	max	67	144	235	1800	1500	9.0

The data in Table 9.3 show that *L. albus* wild and landrace accessions held in the ALC have a wide range of collection site altitudes and indicate that the species can be found up to 2600 m, extending higher than *L. luteus* or *L. angustifolius*. Mean soil pH at collection sites is higher and mean flowering time is lower than in the other two species. On average, accessions were more vigorous at nine weeks after sowing. A wide range of seed weights are available in wild and landrace accessions.

9.4.2.2 Lupinus luteus

9.4.2.2.1 Western Mediterranean

Although it is difficult to clearly ascertain primary and secondary centers of diversity in *L. luteus*, a concentration of genetic variation, including possible wild types, occurs in the high rainfall areas of Portugal, northwest Spain, and Southern Spain. An accession from the latter area was found to have a low branching habit that might have arisen as a result

of selection pressure resulting from grazing animals (Cowling, 2001). Portuguese germplasm includes larger-seeded and early-flowering types and some possibly ancestral, smaller-seeded later-flowering forms, with seed coat of greenish brown on a speckled pale background and pale hilum band (Gladstones, 1998). The brownish seed coat is also found in the eastern Mediterranean (Swiecicki and Swiecicki, 2000). Seeds that are greyish-black are also available and are typically found in the central and western Mediterranean regions. Some accessions from Portugal and the majority of internationally cultivated bitter types have the common dark speckles on off-white background and colorless hilum band. Gladstones (1998) notes similar seed coloration from Calabria in southern Italy and Morocco, as well as Western Australian and South African naturalized populations that are escapes from ornamental or green manuring use there. Swiecicki (1986a) refers to some useful Portuguese landraces with vigor and long main stem racemes setting 6 to 7 whorls of pods. Pereira et al. (2000) demonstrated contrasting groups of Portuguese germplasm from central and southern Portugal. Accessions from Algarve in southern Portugal had early flowering and larger seed. Later flowering germplasm from central Portugal had more branches and longer branches, later flowering, and small seeds. Kurlovich (2002b) states that Iberian yellow lupin germplasm is generally characterized by high biomass, small seeds, and resistance to low temperatures (particularly the Lisbon and Madrid ecotypes) and diseases.

Coastal Morocco provides some very early-flowering and large-seeded material (Clements and Cowling, 1991) and accessions that Kurlovich refers to as the “Rabat ecotype’ and are considered useful as sources of thermoneutrality and high productivity. Naturalized populations from once-cultivated yellow lupin are found in Madeira, the Azores (Papineau and Huyghe, 1989) and southern France (Gladstones, 1974), and these could provide additional variation. Traditional western Mediterranean use for green manuring and forage has apparently given rise to accessions that have high biomass production, many branches, and are frequently late flowering. Gladstones (1974) records that *L. luteus* is distributed in Algeria and western Tunisia, but no information has been found regarding their characteristics. Additional germplasm could be sourced from Corsica, Sardinia, Sicily, and southern Italy (including the islands of Ischia and Capri) (Gladstones, 1974; Swiecicki, 1988a; Kurlovich and Stankevich, 2002). Some southern Italian accessions were described as having many seeds per pod.

9.4.2.2.2 Eastern Mediterranean

Descriptions of genotypes from Israel have ranged from forms with late flowering and small seeds (Kazimierski and Kazimierska, 1975) to those with early flowering and large seeds (Gladstones and Crosbie, 1979; Gladstones, 1998). Kurlovich (2002b) classifies a Palestinian ecotype but subdivides it into two groups: the “Oriental ecotype” (ssp. *orientalis*) with brownish seeds, small, narrow leaflets and short, dense inflorescences and late flowering; and the “Jerusalem ecotype” with black-speckled, large seeds (‘var. *maculosus*’) of up to 160 mg, broad leaflets, early vigor, early flowering, higher yield, with drought tolerance and virus resistance. The author suggests that the larger-seeded ecotype was an introduced form, possibly from European bitter cultivar origins. Some cytological dissimilarity of the Israeli material with that from the western Mediterranean is reported by Kazimierski and Kazimierska (1975). These authors also report Greek and

Turkish *L. luteus*, which was cytologically similar to the western Mediterranean germplasm. Kurlovich (2002b) refers to a "wild form" Anatolian ecotype that is characterized by early flowering, high yield, large seeds (up to 160 mg), and resistance to drought. Swiecicki (1988a) emphasized the importance of Israeli accessions of *L. luteus* for earliness, vigor, large seeds, and brown seed coloration, with material from Lebanon and Syria being a further potentially valuable resource.

Hard-seededness is not a common feature of accessions of *L. luteus* from a range of countries of origin in germplasm holdings in Australia, and this may reflect the scarcity of true wild types. Hard-seeded accessions are represented, however, from Israel, Spain, Portugal, and also from naturalized populations in Western Australia (Smith, 2004). Kurlovich (2002b) mentions seed permeability among *L. luteus* ecotypes, with the Lisbon ecotype having hard seeds and the Madrid and Tangier ecotypes having some hard-seededness. Table 9.3 shows *L. luteus* wild and landrace accessions are found at lower altitudes and have a higher collection site mean rainfall than *L. albus* and *L. angustifolius*. Mean soil pH at collection sites was lower than the other two species. Maximum seed weights only reach 138 mg, and this figure is not exceeded greatly by breeding lines.

9.4.2.3 Lupinus angustifolius

Gladstones (1978) observed a bimodal distribution among 237 Mediterranean *L. angustifolius* of small- (average of 80 mg) and larger- (average of 150 mg) seeded accessions, with overlap in the range of 100 to 120 mg. Similar data are shown by Cowling et al. (1998a). This demonstrated the continuity of variation for seed size and the parallel broader or finer leaf and pod dimensions that exist in collections of narrow-leafed lupin from its natural distribution areas. The smaller-seeded "wild" types tended to originate from more natural, undisturbed habitats, and the larger-seeded types from inland, more fertile soils (often loamy) associated with present or past agricultural or horticultural activity (Gladstones, 1998). Types intermediate between wild and partly domesticated with respect to seed size and plant characteristics (leaflets, height, branching) were found in Iberia, Italy, Turkey, and Israel. These had slightly larger seed size, were not as short or branched, and had larger leaves (Gladstones, 1979). Minor fertility reduction in crosses between wild types from Morocco and the eastern Mediterranean with more domesticated forms probably of Iberian origin does suggest some degree of genetic heterogeneity between germplasm from contrasting backgrounds (Gladstones, 1984). A very broad generalization (with many exceptions) made for *L. angustifolius* provided by Kurlovich (1994) is that duration of the plant growth life cycle becomes longer and biomass increases, while yield, seed size, oil content, leaf size, and drought resistance decrease as origins move from the east to the west Mediterranean. Possible germplasm groups for use in breeding are described here.

9.4.2.3.1 Iberian Peninsula, Morocco, Algeria

Pereira et al. (2000) evaluated Portuguese accessions, both wild and domesticated. They found that wild germplasm characteristically had more leaves on the main stem and smaller seeds than cultivated material, but some wild accessions with few main stem leaves and shorter main stems were from Algarve. Accessions with many main stem

nodes and taller main stems were from Évora. Some wild accessions tending toward larger-seededness were from Alentejo, while the smallest-seeded wild types were from Beira Interior. Gladstones and Crosbie (1979) found some large-seeded strains from inland Portugal (e.g., Évora, Elvas), inland Spain (e.g., Caceres, Ciudad Real, Cordoba, Sevilla), and Morocco (e.g., west of Taza, Chaouen and Al Hoceima region, Tangier and Rabat regions). An unusually large-seeded wild type from Spain found near Cordoba (above 200 mg) was noted by Gladstones and Crosbie (1979) as possibly representing a distinct genetic type. Larger-seeded types from these regions are named by Kurlovich (2002b) as the Iberian roadside, Iberian green manure, and Moroccan roadside ecotypes. That author also describes material from coastal Algeria and Tunisia as thermoneutral, having slow early growth, broad, short leaflets, with seeds ranging to very large in size (130 to 210 mg). In fact, the accession with the highest seed weight in the ALC (above 200 mg), is described as wild and probably originating in Algeria. Other genotypes from Algeria in this collection range from medium large to smaller. Very small-seeded, presumably wild types, were collected from the central Spanish highlands, the sandy coastal lowlands of southern Spain and Morocco, and the Anti Atlas foothills of southern Morocco. Two accessions labeled as *L. opsianthus* (Atabekova and Maissurjan, 1968), (a "wild" form synonymous with *L. angustifolius* and still cited as a different species by some Russian authors) held in the Vavilov Institute collection were collected in Portugal and have been reported to be unique in having low seed coat proportions despite their small seed size (Hauksdóttir et al., 2004).

9.4.2.3.2 Greece, Italy, Eastern Mediterranean

Clements and Cowling (1994) analyzed collections from the Aegean region (Cowling, 1986) and found a particularly high degree of genetic variation associated with the wide range of geographic and climatic conditions represented. Using 19 morphological traits taken on 157 accessions, they identified 13 groups based on hierarchical cluster analysis. Fine-leafed, small-seeded, later flowering types were mainly found at higher altitudes of northern Greece and some islands. Types with large leaves, pods, and seeds were found at lower altitudes as volunteer weeds and sometimes on fertile soil. The results supported the suggestion that larger-seeded types were selected in arable areas for food, green manure, or forage (Gladstones, 1998). Two of the groups from the Dhodhekanisos Islands highlighted valuable agronomic types with large seeds and many main stem pods. Swiecicki (1988a) highlighted the Apeninian Peninsula and surrounding islands (Italy, Sicily, Sardinia, and Corsica) as one of the richest regions of lupin diversity, with particular concentration in the triangle formed by Rossano, Cosenza, and Reggio di Calabria. There are five lupin species represented there. Germplasm of narrow-leafed lupin from areas including Mt. Etna (Sicily), the islands of Ischia, Capri (Italy), Corsica, Sardinia, Cyprus, and Syria could provide useful variation. Some accessions of *L. angustifolius* (as in *L. luteus*) had a high number of seeds per pod (Swiecicki, 1988a). Another region that may provide some uniquely adapted germplasm includes the inland basalt soils of Israel, which were described by Plitmann (1966) and Pazy et al. (1977) as the var. *basalticus* type and types with very narrow and relatively short leaflets growing mainly on the coast of Israel (Plitmann and Heyn, 1984).

Table 9.3 shows that, although mean soil pH at collection sites was 6.7, maximum pH values reach 9. An accession (P22872) from Morocco was collected on a red-brown clay loam soil of pH 8 and is the parent in cvv. Belara and Yorrel. These cultivars tend to be more adapted to duplex and heavy soils in Western Australia, and part of this may be attributed to the wild types characteristics. A large range of seed weights are available for *L. angustifolius* (Table 9.3).

9.4.2.4 Other Smooth-Seeded Old World Lupins in Relation to Crop Improvement

Lupinus hispanicus germplasm consists of the two subspecies, *hispanicus*, having violet flowers and larger seeds, and ssp. *bicolor*, with cream and yellow becoming lilac flowers, and smaller seeds. *Lupinus hispanicus* ssp. *hispanicus* is found mainly in southern and central Spain and ssp. *bicolor* at higher elevations in cool, moist areas of northwest Spain and rarely in northern Portugal. White or pale cream flowering plants that become more or less dark crimson have been reported (Cordero et al., 1988; Cowling et al., 1998a) that are possible intermediate forms between the two subspecies in their region of distribution overlap in central Spain. Gladstones (1974) notes populations displaying possible introgression between ssp. *hispanicus* and *bicolor* and between ssp. *bicolor* and *L. luteus*. Cordero et al. (1988) and Arrieta et al. (1994) describe germplasm with variation for characters such as “rough” and “smooth” seed coats, varying leaflet numbers of the first leaf pair, alkaloid contents and profiles of light-yellow vs. white flowered plants (especially with regard to epilupinine and lupinine), and seed coat permeability. They identified naturally occurring genotypes from two origins that had permeable seed coats. Possible occurrences of *L. hispanicus* in Turkey and Northern Greece, as noted previously, may provide additional genetic diversity.

The ALC holds accessions of *L. micranthus* representing Spain, Portugal, Morocco, Italy, Greece, Israel, and the former Yugoslavia (Smith, 2004). Gladstones (1974) lists occurrences on neutral and calcareous soils and soil textures ranging from sandy to clay loam. Its use in classical times as a green manure plant in Italy, Greece, and the islands of Dalmatia is also mentioned (Gladstones, 1974). Seed sizes ranged from 40 to 139 mg, and it is suggested that the larger types may have resulted from human selection.

9.4.3 Genetic Diversity of the Rough-Seeded Species

Among the rough-seeded lupins, *L. pilosus* has the widest range of flowering times, with some accessions flowering as early as 82 days and others as late as 132 days (Table 9.4). *Lupinus atlanticus* and *L. cosentinii* also have large ranges in flowering time. The large range in flowering time for *L. pilosus* and *L. atlanticus* might be the result of these species growing across a wide range of altitudes. However, flowering time and altitude are not directly related, since in *L. atlanticus*, the northern population, which is the latest flowering, comes from the middle of the range in altitude. *Lupinus princei* has the longest time to flowering of all the rough-seeded lupin species. The length of the

Table 9.4 Ranges of Days to Flowering, 100 Seed Weight, Collection Site pH, and Collection Site Altitude for the Rough-Seeded Lupin Species Held in the Australian Lupin Collection

	L. pilosus	*L. atlanticus*	*L. cosentinii*	*L. palaestinus*	*L. princei*	*L. digitatus*
Days to Flowering	82–132	93–133	87–134	81–103	135–150	102
Seed Weight	32.3–76.1	20–52	5.8–23.3	19–26	33	12
Collection Site pH	5.5–9.5	6.5–9.5	6–9	7.7	5.7	
Collection Site Altitude (m)	3–1100	460–1630	3–800		2100–2460	

flowering time makes it unsuitable for cultivation in a Mediterranean type climate. *Lupinus palaestinus* has a small range of flowering times, possibly reflecting the narrowness of its habitat. The rough-seeded lupins are mostly associated with alkaline soils (Table 9.4). *Lupinus pilosus, L. atlanticus,* and *L. cosentinii* are found on soils that are slightly acid (6.0) to highly alkaline (9.0). *Lupinus princei* was found on soils of pH 5.5, while *L. palaestinus* comes from alkaline soils (8.0). No pH data have been collected for *L. digitatus,* although it has been reported to come from calcaric fluvisol-type soils. The soils of the Nile Valley, where it used to grow, are highly alkaline (pH 9.5).

Lupinus cosentinii is predominantly found in the lower altitudes along the coastal plains (Table 9.4). There have only been a few collections from higher altitudes in Morocco. *Lupinus pilosus* is found from the low coastal altitudes to the higher altitudes in the mountains, especially in Crete. *Lupinus atlanticus* only grows in the mountainous areas of Morocco at altitudes between 450 and 1650 m. *Lupinus palaestinus* grows in coastal plains and into the mountains but would only be found at altitudes less than 1000 m. *Lupinus princei* is found in the eastern highlands of Africa at high altitudes. It is found on Mt. Kenya at 2200 to 2600 m, making it the rough-seeded lupin that grows at the highest altitude. *Lupinus digitatus* is associated with the mountainous regions of the southern Sahara and the valleys of the Nile and Senegal rivers, so it can grow over a range of altitudes. *Lupinus pilosus* covers a range of seed sizes from 32.3 to 76.1 mg per seed (Table 9.4), nearly as wide as the range for *L. albus*. Within that range, there is also considerable variation in seed coat color and markings, and it includes a small number of accessions that have smooth seed. *Lupinus atlanticus* seed size varies from small types in the south weighing 20 mg to the larger-seeded types in the north at 52 mg per seed. *Lupinus cosentinii* has a wide range of seed types as well with some seed as small as 5.8 mg. *Lupinus palaestinus* and *L. princei* have limited collection data but fall between 20 and 40 mg per seed. The seed size for the one collection of *L. digitatus* is 12 mg per seed.

The literature mentions *L. digitatus* having large seeds, so this one collection may be from the lower end of the range.

9.4.3.1 Intraspecies Diversity

9.4.3.1.1 Lupinus atlanticus

Lupinus atlanticus is found in four main areas in the Atlas and Anti-Atlas mountains of Morocco. These are designated as south (including those centered around the village of Tanalt and Tafraoute), central (centered around the village of Amizmiz on the western slopes of the foothills of the Atlas Mountains), valley (the deeper valleys running into the Atlas Mountains), and north (centered around the city of Beni Mellal). While these are arbitrary regions, they define distinct areas where *L. atlanticus* is found. The main feature dominating these distinctive areas apart from geographical location is that they fit into fairly distinct rainfall areas and at distinct altitudes. Based on these two measurements alone, the accessions separate into those designated regions. Upward of 20 different phenotypic traits were measured on 54 accessions of *L. atlanticus* collected from the Atlas and Anti-Atlas mountains of Morocco. There were four distinct regions where *L. atlanticus* was collected—north, central, valley, and south. Principal coordinate analysis of these data resulted in the northern group being easily distinguishable from the other three groups. Because of the large variation between accessions within these other groups, they could not be separated from each other and overlapped considerably. Accessions from the northern region had early rapid growth with maximum values for growth, flowered late, and had exceptionally large seed. The accessions from the valley region were slow growing (minimum values), late flowering, and had the highest number of pods on the main stem. The accessions from the southern and central regions had similar growth characteristics, flowered earlier than the other two regions, and had larger leaves. Principal coordinate analysis (PCA) indicated large variation within the different groups and was able to clearly separate the northern populations from the other regions. There was too much variation in the individual accessions from the other regions for them to be clearly separated from one another. Epilupinine and multiflorine are the two dominant alkaloids in *L. atlanticus*. The ratio of these two alkaloids change from the northern populations (0.72), where multiflorine is the highest, to the south (1.19), where epilupinine is the highest. The total alkaloids varies from 0.25 to 0.50% dry weight.

9.4.3.1.2 Lupinus pilosus

Lupinus pilosus grows in a wide range of environments and on different soil types. This has resulted in the species having a great diversity in genotypes across its habitat range. Flowering times range from 82 to 132 days. The early-flowering types usually have early rapid growth and require no vernalization for flowering. The late-flowering types require vernalization and tend to have a long period in a rosette state before rapid growth at flowering. The seed size varies from small (42 gm/100 seeds) to very large seeds (72 gm/100). Clements et al. (1996) examined 71 accessions of *L. pilosus* for variation in morphological characters. Using hierarchical cluster analysis, they were able to choose

10 groups, which accounted for 79% of genotype sums of squares and 69% of genotype times character sums of squares. These 10 groups fell into 4 different categories: Group 132, which was distinguished by poor pod set, few seeds, and small leaves. The plants were poorly nodulated and had pale yellow leaves. This group was easily distinguishable from the other accessions of *L. pilosus*. Pod set was improved with artificial tripping flowers. Interestingly, the receptacles on stigmas were very small and the peristigmatic collar had long epidermal hairs. This is a similar characteristic to *L. palaestinus*, and this group may either represent a distinct variation of *L. pilosus* or hybrid swarms of *L. pilosus/L. palaestinus* hybrids, although the latter may not be the case since *L. palaestinus* is restricted to the coastal areas of Israel. All the members of this group came from northern and eastern Israel. The second category was formed by Groups 126, 128, and 130. These groups had short early growth, late flowering, and short mainstem inflorescences. The groups were also very fertile with large numbers of pods on the main stem and many seeds per pod. The late-flowering group 128 from Crete was highly branched. Group 130 had large leaves and seed and originated at moderate to high altitudes in Greece, Syria, and Israel. The third category of groups (125, 127, 129, and 131) included accessions that had early rapid growth and early flowering. Group 127 included most of the botanical garden selections from Europe and Australia. Groups 125, 129, and 131 included the accessions from Greece, Syria, and Israel that had rapid early growth and early flowering. Group 129 had accessions with the largest leaves and seeds. The fourth category included Groups 107 and 111, which had long mainstem inflorescences. These two groups were dissimilar in other characters. Group 107 was closer aligned to Group 132, with poor pod set and low yield. Group 111 was late flowering and very rosetted in the early stages of growth. The accessions in Group 111 were collected in Turkey, had the smallest seeds of all the accessions, and came from high altitudes.

Within *L. pilosus*, there have been three accessions found with smooth seed. Two accessions have all the seed smooth while the third had a mixture of smooth and rough seed. All these accessions were collected in the Tartus and Homs regions of southern Syria. A Polish group has collected a smooth-seeded lupin from Turkey. While they have suggested that this may be a new species of lupin, the evidence seems to be that the smooth seed coat is only a variation within *L. pilosus*. The smooth-seeded types are very similar in plant structure to other rough-seeded plants from the same area.

9.4.4 Genetic Diversity of *Lupinus mutabilis*

The existence of wild forms of *L. mutabilis* is contentious, with some authors reporting their occurrence in the traditional areas of cultivation in the highlands of Bolivia, Peru, and Equador (Blanco, 1982), and others tending to believe existing populations are not true wild types (Gonzalez, 1986). Wild plants are described as having small leaves, with narrow leaflets, small pigmented pods that are fully dehiscent, and seeds are small (5 to 7 mm), black or marbled, and impermeable to water (Blanco, 1982). We have seen characters possibly representing the wild state: purple flowers with a yellow or white spot on the standard, highly pigmented stems, pod shattering, prostrate growth habit. Some ecotypes in the Bolivian highlands with small seeds and shorter growth habit may be of this wild type. Tapia and Vargas (1982) referred to several species as possible wild

relatives of *L. mutabilis.* These were *L. praestabilis* (very similar to *L. mutabilis*), *L. aridulus, L. biinclinatus, L. calcencis, L. condensiflorus, L. cuzcencis, L. malacotrichus, L. microphyllus, L. paniculatus, L. praealtus, L. semiprostratus, L. chlorolepis,* and *L. tomentosus,* several of which are given the name "ckera" or "kita" by Quechua people, referring to wild lupin species. They were recommended for introgression to provide frost and anthracnose resistance for *L. mutabilis*. Planchuelo (1994), however, stated that after preliminary taxonomic studies, these species were not as closely related to *L. mutabilis* as was originally proposed. *Lupinus cruckshanskii* is another species that has been identified to be genetically close to *L. mutabilis,* based on ITS sequences (Käss and Wink, 1997b).

Lupinus mutabilis germplasm includes the annual and biennial, plants with long branches adapted to longer growing seasons of up to 325 days in the Andes and the shorter season types from high elevations in the central Andes. Seed color of germplasm was white in 95% of the samples studied by Blanco (1982). Shorter growth habit plants with a dominant main stem and few branches were reported to exist in the high altitude region around Lake Titicaca, a region with low minimum temperatures that might also provide genotypes with tolerance to frost. Landrace selections from southern Peru and southern Bolivia are noted as being earlier maturing, while Ecuadorian material had high yield potential (von Baer and von Baer, 1988). Several early-flowering accessions were also reported to originate from Peru (Neves-Martins, 1994), and the early selection named Potosi originated in Bolivia (Römer and Jahn-Deesbach, 1986).

With *L. mutabilis'* natural adaptation to altitudes between 2500 and 3800 m (and cultivation up to 4000 m) in the Andes, it is subjected to a temperature range that may restrict genetic variability of germplasm with respect to temperature adaptation to other regions. Where *L. mutabilis* grows in the Andes regions of Colombia, Equador, Peru, and Bolivia, the temperature range during the year is relatively narrow, with average daily temperatures during the year reducing to approximately 7°C in some sites and extending to 27°C in others (Gonzalez, 1986). Many localities, however, have much narrower ranges of average daily temperatures, frequently only varying by 10°C year round. For example, the Columbian highlands have an average temperature range from 21 to 27°C, with precipitation of 1200 to 1500 mm. The highlands of Peru and Ecuador have a temperate climate, with average temperatures ranging from 10 to 16°C and 750 to 1000 mm rainfall. In the Bolivian highlands, agricultural activity occurs in the irrigated valleys, such as Cochabamba, and in the lower rainfall high plains of the Andean central region. Rainfall averages from 660 to 1200 mm, and most of this falls from December to January. Dunn (1984) reports a general requirement among the majority of New World species, including *L. mutabilis*, for a night temperature below 16°C. *Lupinus mutabilis* is described as photoperiod neutral (Hackbarth, 1936), intolerant to frost, particularly at the time of sowing, having a preference for soil pH in the range of 5 to 7, with growing season lengths in its natural environment of from 5 to 10 months, and a growing period from spring sowing in September to November to harvest in the dry winter (June to August) (Gonzalez, 1986; Mujica, 1994; Mujica et al., 2004). Reports of susceptibility to frosts of −2°C at the flowering and podding stage also exist (Tapia and Vargas, 1982). Soil types in its natural distribution include primarily inceptisols with some areas of oxisols and ultisols. Water requirements for the growing cycle have been estimated to be from 350 to 800 mm (Mujica, 1994), with a more recent estimation from 570 to 800 mm (Mujica, 2004). From these figures and the findings of other studies indicating its

susceptibility to frosts (Lopez Bellido and Fuentes, 1990), the species is not generally as drought or cold tolerant as, for example, *L. angustifolius*.

Among *L. mutabilis* germplasm held in the ALC, a useful range of plant characters are available in germplasm consisting of "wild" or landrace accessions (from Peru, Bolivia, Ecuador, and

Table 9.5 Variation for Plant and Seed Traits in *Lupinus mutabilis* Wild and Landrace Accessions, and Breeding Lines or Mutants in the Australian Lupin Collection

	Wild Types and Landraces				Breeding Lines, Selections, Mutants			
	n	min	mean	max	n	min	mean	max
Days to Flowering	33	55	88	114	63	65	89	114
Seed Weight (mg)	31	68	186	255	62	103	157	246
Seed Coat (%)	36	10.6	12.9	16	63	11.3	12.6	15.6
Pod Wall (%)	17	35.8	45.1	52.6	9	39.4	44.7	53.1
Seeds per Pod	16	2.7	4.4	5.5	9	3.1	5.1	6.8

Argentina) and breeding lines, selections, and genotypes from a mutation program of Pakendorf (1974). A full range of days to flowering and seed size has been found with potentially exploitable variation for other characters such as seed coat and pod wall proportion and seeds per pod (Table 9.5). Seed shape variability has been observed among different accessions that range from large and almost spherical to round-oval or tear-drop shaped, and also flattish. Flower colors include purple, purple/yellow, purple/white/yellow, blue/yellow, blue/white/yellow, white/yellow, pink/white/yellow, pink, and white. Seed colors vary from black, marbled, white with black hilum, crescent or eyebrow, and fully white.

9.4.5 Priorities for Collection and Conservation of Old World Lupins and *Lupinus mutabilis*

Although the *ex situ* stocks of lupin accessions are relatively high, there are a large number of duplications in genebanks that have arisen as a result of exchange between institutions. Accession holdings can sometimes be exaggerated by including selections made from the same originally introduced accession. *In situ* wild types and landraces distributed in many regions of the Mediterranean and South America are at risk due to increasing human activity. A number of collectors have noticed the genetic erosion of lupin genetic resources occurring in many countries. Grazing pressure in certain regions of Morocco and Ethiopia, and the rapid disappearance of wild and landrace lupins from areas such as the Golan Heights in Israel is of concern for the future collection of lupins from these areas (Francis, personal communication, 2004). Cowling et al. (1998b) reported the dramatic reduction of landrace populations of *L. albus* in the Azores Islands

since the collections of Papineau and Huyghe (1989). There is a need in South America to catalog all of the genetic resources held in South American countries to identify regions for further collection. There might still be a need for long-term seed storage facilities in these countries (Cowling, 1998a). More efficient exploitation of germplasm in breeding programs can be promoted by the development of better screening methods and international collaborative evaluation for pests, diseases, and other traits. Development of core and mini-core collections as has been achieved, for example, in chickpea and groundnut (Upadhyaya and Ortiz, 2001; Upadhyaya et al., 2002a, 2002b, 2003) is recommended, both using morphological traits and molecular methods. Based on known natural distributions of species, reported holdings of accessions, and other published reports, priorities for lupin genetic resource conservation include the following.

9.4.5.1 Lupinus albus

Lupinus albus var. *albus* from Verde Islands, Calabria (Italy), Sicily (e.g., from the valley of San Marteno, near Palermo), Malta, Corsica (e.g., northern part), Bulgaria, Romania, Hungary, Albania, Yugoslavia, Turkey, Sudan, Kenya, Syria, Lebanon, Israel, Morocco, Algeria and Tunisia; *L. albus* var. *graecus* from as many regions as possible, i.e., Turkey, Greece, Albania, Yugoslavia; collection for cold and frost tolerance from higher altitudes or latitudes; anthracnose, Fusarium wilt resistance.

9.4.5.2 Lupinus luteus

Wild types in the high rainfall areas of Portugal, northwest Spain, southern Spain. Landraces from Israel (large-seeded types, particularly), Italy, Sardinia, Corsica, Morocco, Algeria, and western Tunisia. Collection for cold and frost tolerance possibly from higher elevations in Spain and Portugal; anthracnose and fusarium wilt tolerance and virus resistance, particularly bean yellow mosaic virus (BYMV).

9.4.5.3 Lupinus angustifolius

South and western France, Morocco (e.g., Amizmiz, Demnate, Anti-Atlas Mountains, northwest of the Atlas Mountains), Algeria, Tunisia, Italy, including Sicily (e.g., Mt Etna), *'L. linifolius'* types, islands of Ischia and Capri, Corsica, Sardinia, Cyprus, Turkey (including islands), Syria, Lebanon, Israel including inland basalt soils, Egypt; exploration of Greek islands such as Ionian Islands, Amorgos, Astipalaia, Milos, Serifos, Kithira, Rhodes, Karpathos, Lesbos; *'L. opsianthus'* types from Portugal; collection for anthracnose and fusarium wilt resistance; collection of *L. angustifolius* with tolerance to heavier textured or alkaline soils.

9.4.5.4 Lupinus micranthus

As many regions around the Mediterranean as possible, including Portugal, Morocco, Menorca, Corsica, Sardinia, Italy, Turkey, Cyprus, Syria, Lebanon, Israel.

9.4.5.5 Lupinus hispanicus

Turkey and Northern Greece.

9.4.5.6 Rough-Seeded Lupins

All of the individual species in the group are in great need of further collection to preserve the genetic diversity that exists between and within the species.

9.4.5.6.1 Lupinus mutabilis

Documentation and mapping of existing world-conserved genotypes of *L. mutabilis* and determination of collection gaps in South America; collection from the west side of the Andes, highlands of Huanuco and Chachapoyas in Peru (Cowling et al., 1998a); collection targeting greater, morerapid leaf area development; low-temperature tolerance from regions of high altitude; targeting types with lower pod wall proportion and higher harvest index; resistance to anthracnose, brown leaf spot, *Pleiochaeta* root rot; tolerance to alkaline soils; collection of species possibly closely related to *L. mutabilis;* collection of naturally occurring low alkaloid types, e.g., from Ecuador and Peru.

9.4.5.6.2 Rhizobia

Collection and conservation of *Rhizobia* associated with lupins from different geographical regions to target different elevations, soil types, and pHs; examples of this are collection of isolates from alkaline soils (e.g., Egypt, Morocco, Israel, Syria); from very low pH soils (e.g., Portugal and Spain) or high aluminum soils; cold-tolerant strains from high altitude sites in the Andes or from *Lupinus* species in the arctic regions; research in other species has shown that artic rhizobial strains were more effective for nitrogen fixation at low temperatures than strains from temperate regions (Prevost and Bromfield, 1991); variation for strain effectiveness has been reported for performance of *L. nootkatensis* in Iceland (El-Mayas, 1999).

9.5 GERMPLASM ENHANCEMENT

9.5.1 Conventional Breeding

The history of lupin breeding can be found in Gladstones (1970), Hondelmann (1984), and Cowling et al. (1998b), with more specific discussion of Australian lupin breeding in Gladstones (1994), and German work in Hondelmann (2000) and Brummund (2000). A summary of the major steps in lupin crop development and plant breeding is given in Table 9.6. The identification of natural mutants of lupins with low alkaloids by Reihold von Sengbusch, and subsequently by others, marked the beginning of modern lupin breeding. These simply inherited genes for reduction of alkaloid included *dulcis (dul)* and *amoenus (am) (L. luteus), iucundus (iuc) (L. angustifolius),* and *pauper (pau), mitis (mit),*

nutricus (nut) (L. albus). The unprecedented first steps in the transformation from a wild plant to a domesticated crop with good biological and seed yields, serving as one of the best plants for nitrogen supply in rotation on infertile soils, was attributed in the three decades following to German breeders von Sengbusch, Hackbarth, Troll, Heuser, and Kess. Following the selection for low alkaloid was the incorporation of permeable seeds in *L. luteus* and *L. angustifolius* lupin by von Sengbusch and Troll and nonshattering pods in *L. luteus* by von Sengbusch and Zimmerman. The identification of the gene *mollis (moll)* for impermeable seeds in *L. angustifolius* was reported by Mikolajszyk in 1966 and Forbes and Burton in 1968 (Gladstones, 1970). The combining of white seeds and nonshattering pods in *L. angustifolius* came when Gladstones discovered natural mutants in cv. New Zealand Blue with the gene for white flowers and seeds *(leucospermus)* and the genes for reduced podshattering *lentus (le)* and *tardus (ta)* by the early 1960s (Gladstones, 1967). With the identification of earlier-flowering natural mutant genes *efl* and *Ku* (Gladstones and Hill, 1969), *L. angustifolius* had become a truly domesticated crop plant. Early breeding methods mainly involved plant selection following simple hybridization between natural or induced mutants with bitter varieties and landraces. After this initial period of combining simply inherited major traits from natural mutants came selection through recombination breeding for more complex traits such as adaptation and yield, and for the successive changes in traits such as flowering time. Mutation breeding was also used, especially in countries such as Poland and Russia, to introduce traits such as restricted branching. The four major lupin crop species each have relative general differences, although often overlapping plant adaptation and seed quality characteristics (Table 9.7), and breeders have focused on enhancing or broadening these within the genetic limits of the germplasm base.

9.5.1.1 Lupinus albus

Lupinus albus is recognized for its generally wide adaptation and good seed quality. Seed protein content varies from 33 to 47% and oil content from 6 to 13%, depending on genotype and environment (Petterson et al., 1997; Huyghe, 1997). Although predominantly self-pollinating, outcrossing rates of 8% (Faluyi and Williams, 1981) to 10% (Green, 1980) are reported for this species, with some indications of better pod setting when flowers are tripped. Breeding of *L. albus* has been conducted typically through pedigree selection of pure lines. Isolation distances of approximately 35 m gave outcrossing rates of 0.04% (Faluyi and Williams, 1981). Other reports recommend that seed multiplication with a 300 m isolation distance be used (Huyghe, 1997). Le

Table 9.6 Significant Events in Lupin Crop Development and Breeding

Date	Events	Varieties or Lines from That Period
Ancient or Pre-1700s	*L. albus and L. muta*bilis non-shattering, permeable and white seed coat genotypes already available, *L. angustifolius*-large seeds, rapid growth	
1780	*L. albus* imported from Italy into Prussia, scattered cultivation followed	
1860s	*L. luteus and L. ang*ustifolius cultivation in Germany/Baltic coastal plain for green manure and grain for sheep	
	Became important part of merino wool industry of Saxony; *L. angustifolius* used in Suffolk	
	L. cosentinii introduced into Western Australia to become naturalized	
1870s	Experiments by A. Schultz-Lupitz encouraged use for soil fertility improvement; Lupinosis occurrences	
1900s	Decline in wool industry and imports of nitrogen fertilizers reduced lupin area in Germany	
1920s	Post-war interest in protein feeds, alkaloid extraction methods devised	
	E. Baur (Director Kaiser Wilhelm Inst. for Breeding Research, Berlin) proposed low alkaloid mutants possible	
	Bitter varieties of *L. albus, L. luteus, L. angustifolius* selected for vigor and ealier maturity in north European summer	Pflugs Allerfrüheste *(L. angustifolius)*
1928–1929	Development of iodine-mercury-potassium iodide solution to distinguish low alkaloid in lupin; E. von Sengbusch (Germany) selected first low alkaloid natural mutant plants of *L. luteus, L. angustifolius*	Müncheberger Blaue Grünfutter Süsslupine *(L. angustifolius),* Müncheberger Gelbe Grünfutetrsüsslupine St. 8 *(L. luteus)*
1930s–1940s	Fully domesticated lupin: nonshattering pods in *L. luteus*-von Sengbusch & Zimmerman; permeable seeds in *L. luteus*-von Sengbusch; white seeds in *L. luteus*-H. Troll; Low alkaloid plants in *L. albus, L. mutabilis*-von Sengbusch and Heuser, and in *L. angustifolius, L. luteus, L. polyphyllus*-Fedotov and Ivanov (Russia); permeable seeds in *L.*	Nährquell, Kraftquell *(L. albus);* Yubileiny, Weiko II *(L. luteus);* Müncheberger Blaue Süsslupine II, Blusa 3, Borre *(L. angustifolius)*

	angustifolius-A. Hagberg-(Sweden), and permeable seeds and early vigor in *L. angustifolius*-H. Troll (Germany)	
1950s	Anthracnose, grey leaf spot resistance in *L. angustifolius*-J. Weimer, I. Forbes, H. Wells (U.S.); early vigor, light yellow flowers in *L. luteus*-J. Hackbarth (Germany), fusarium and mildew resistance in *L. luteus*-H. Lamberts (Netherlands), early maturing *L. luteus*-O. Tedin & A. Hagberg (Sweden); thermoneutral *L. albus*-J. Hackbarth, W. Plarre; early flowering in *L. cosentinii*-J. Gladstones & G. Hill	Gülzower Süsse Gelblupine; Weiko III, Palvo, Sulfa, Alteria, Schwako *(L. luteus);* Neutra, Ultra, Hansa, Blanca *(L. albus);* Gülzower Süsse Blaue Lupine, Grisa *(L. angustifolius);*
1960s	Very early *L. luteus*-G. Taranukho (Russia), restricted branching *L. luteus*-V. Valovnenko (Russia), restricted branching recessive natural mutant in *L. luteus* (ex Hungary 1950s)-H. Troll (Germany); *L. angustifolius*-permeable seed coat, white flowers and seeds, cold tolerance-I. Forbes; nonshattering pods, moderately early and early flowering (no vernalization requirement) from cv. Borre in *L. angustifolius*-J. Gladstones (Australia); self-completing *L. albus*-Kazimierski & Swiecicki, J. Mikolajczyk (Poland); early flowering and nonshattering pods in *L. cosentinii*-J. Gladstones & C. Francis	Academichesky 1, Zhitomir anniversary, Zhitomirskaja jubeljenaja, Refusa, Bas *(L. luteus)',* Blanco, Rancher, Uniwhite, Tiftblue-78, Frost *(L. angustifolius);* Wat *(L. albus);* Chapman *(L. cosentinii)*

Date	Events	Varieties or Lines from That Period
1970s	Fully domesticated *L. angustifolius,* combined grey leaf spot and anthracnose resistance-J. Gladstones, restricted branching (natural and induced mutants) in *L. angustifolius*-Russia, Poland, I. Forbes (USA); very early flowering and restricted (determinate) branching in *L. albus, L. angustifolius*-V. & O. Golovchenko, G. Gataulina, N. Maisurjan, N. Pukhalskaja, N. Klochko and others (Russia and Poland); cold tolerance in L. albus-H. Wells, I. Forbes (USA); wider soil adaptation in *L. luteus*-H. Troll, further fusarium resistance in *L. luteus,* increased yield from low alkaloid types, thermoneutral (from wild sources) *L. luteus*-W.K., W. Swiecicki (Poland), restricted branching *rb* gene in *L. luteus*-Svab (Hungary); stable bitter cultivars from ecotypes of *L. mutabilis*-G. Blanco (Peru), low alkaloid *L. mutabilis* lines-E. von Baer & R. Gross (Chile), K. Pakendorf (South Africa), early flowering *L. mutabilis*-M. Lenoble (France), male sterility in *L. mutabilis*-K. Pakendorf	Rufusa nova, Borluta, Topaz, Tomik, Palucki, Afus *(L. luteus);* Neuland, Start, Kiev Mutant, Bialy 7, Gorizont, EP-1, Giza 1, Tiftwhite *(L. albus);* Uniharvest, Unicrop, Marri, Illyarrie, Northern 3, Timir-1, Ladny, Lanedeks-1, Mut-1 *(L. angustifolius);* Erregulla *(L. cosentinii)*
1980s	Restricted branching spring *L. albus* mutants-	Inti, Potosi *(L. mutabilis);* Erregulla

	Mikolajczyk (Poland), identification of seed coat and pod wall thickness variation in *L. albus*-M. Lenoble (France); Early flowering *L. mutabilis* - Römer & Jahn-Deesbach (Germany), W. Williams (UK), J. Rivero (Peru), low alkaloid *L. mutabilis* cultivar; Phomopsis resistance in *L. mutabilis*-Van Jaarsveld & Knox Davies (South Africa); fully domesticated *L. cosentinii*-J. Gladstones; further fusarium resistant *L. luteus, L. angustifolius*-M. Lukashevich, N. Kuptsov (Byelorussia), Brummund (Germany); Phomopsis resistance in *L. angustifolius*-J. Gladstones, W. Cowling, M. Sweetingham (Australia), improved agronomic packages assisted adoption and yield increases in Australia; quantitative resistance to bean yellow mosaic virus in *L. luteus*-H. Schmidt (Poland)	Soft *(L. cosentinii);* Zhodinsky, Bornova, T-1, S-1, Boresa, Ventus *(L. luteus);* Gungurru, Galena *(L. angustifolius);* Lucky, Lublanc, Lutop *(L. albus)*
1990s	Dwarf mutants in *L. albus*-R. Muranyi & E. Sawicka (Poland), C. Huyghe & N. Harzic (France); Exploiting plant architectures in *L. albus*-C. Huyghe, B. Julier (France), G. Milford (UK); Further development of restricted branching (determinate) cultivars of *L. luteus*, cucumber mosaic virus (CMV) resistance identified in existing Polish cultivars of *L. luteus*-R. Jones (Australia); higher harvest index, better anthracnose and *Pleiochaeta* resistance cultivars of *L. angustifolius*-J. Gladstones, W. Cowling, M. Sweetingham; restricted branching research in *L. angustifolius*-M. Dracup (Australia); early and restricted branching mutants of *L. mutabilis*-Sawicka-Sienkiewicz (Poland), restricted branching *L. mutabilis*-P. Römer (Germany); domestication of rough-seeded lupins-B. Buirchell (Australia); genetic transformation of *L. polyphyllus, L. hartwegii*-J. Berlin et al. (Germany) (?), *L. angustifolius*-A. Pigeaire, C. Atkins et al. (Australia), L. Molvig et al. (Australia), *L. albus*-Guines et al. (France)	Borselfa, Borsaja, Manru, Radames, Markiz, Polo, Teo, Lidar, Wodjil *(L. luteus);* Merrit, Tanjil, Belara, Quilinock, Myallie *(L. angustifolius);* XA100 *(L. albus;);* Mut-136, KW *(L. mutabilis).*
2000	Molecular markers for anthracnose resistance in *L. angustifolius, L. albus*-H. Yang (Australia); anthracnose resistance in *L. albus* landraces-C. Francis and others (Australia); genetic transformation of *L. luteus*-Li et al. *L. mutabilis*-Babaoglu et al.; rapid seed filling identified in *L. angustifolius*-J. Palta (Australia): CMV resistance in L. *mutabilis*-R. Jones (Australia)	Legat *(L. luteus);* 'Mandelup' *(L. angustifolius).*

Source: From various sources, including Gladstones (1970, 1994), Hondelmann (1984,2000), Brummund (2000).

Table 9.7 General Characteristics of the Major Crop Lupin Species

	L. albus	*L. luteus*	*L. angustifolius*	*L. mutabilis*
Growing Season Climate	Cool to moderately warm, some frost tolerance	Mild temperatures, frost susceptible	Cool to mild temperatures, moderately tolerant to frost	Narrow temperature range, very frost susceptible
Soil Adaptation	Mildly acid to mildly calcareous loamy sands and loams, very intolerant of waterlogging, proteoid roots give efficient P uptake, low cadmium accumulation	Strongly to mildly acid sands and sandy loams, some waterlogging tolerance, aluminium tolerant, sensitive to alkaline soils, cadmium accumulator, more effective uptake of P and Zn than *L. angustifolius* (third order lateral roots)	Moderately acid to neutral sands and sandy loams, intolerant of waterlogging, low cadmium accumulation	Mildly acid to neutral loamy sands and loams, tolerant of waterlogging, low cadmium accumulation
General Soil Fertility Requirement	Moderate	Low	Low to moderate	Moderate
Water Requirement	Moderate	Low to moderate	Low	Moderate
Fungal Diseases	Very susceptible to anthracnose; susceptible to fusarium, rust, botrytis; resistance to phomopsis generally although susceptibility reported in S. Africa	Susceptible to anthracnose single gene resistances available for fusarium; resistant to Pleiochaeta root rot and good sources of resistance to brown spot; moderate resistance to phomopsis	Susceptible to anthracnose, moderate resistance available (*An* and other genes); susceptible to fusarium; susceptible to Pleiochaeta root rot and brown spot but polygenic resistance available; susceptible to phomopsis but resistance available *(Phr1, Phr2)*	Susceptible to anthracnose but less so than *L. albus;* resistance available to fusarium; very susceptible to Pleiochaeta root rot and brown spot but some genetic variation; relatively resistant to phomopsis
Virus Diseases	CMV: tolerant; BYMV: moderate problem and seed borne in east and central Europe.	CMV: susceptible; serious problem in east and central Europe—carried over through lupin seed; resistance to	CMV: highly susceptible; serious problem, seed-borne, partial resistance to seed transmission used in breeding in	CMV: susceptible; moderate problem, seed transmission not recorded, resistance found in one line

	and U.S.; not seed-borne in Australia. A non-necrotic strain causing concern in Australia	seed transmission occurs; single gene resistance *Ncm-1* BYMV: serious problem in east and central Europe, and in U.S.—carried over through lupin seed; resistance to seed transmission occurs; partial resistance to BYMV infection by aphids used in breeding in Europe; very low alkaloid lines susceptible to aphids	W. Australia BYMV: serious problem, high susceptibility, not seed borne, partial resistance to infection by aphids occurs; two strains, necrotic and non necrotic	BYMV: highly susceptible
Herbicide Tolerances	Moderate tolerance to simazine and diflufenican, susceptible to metribuzin, tolerant to grass-selective herbicides	Moderate tolerance to simazine and diflufenican, susceptible to metribuzin, tolerant to grass-selective herbicides	Tolerant to simazine and diflufenican, some cultivars tolerant to metribuzin, tolerant to grass-selective herbicides	Moderate tolerance to simazine, susceptible to metribuzin, tolerant to grass-selective herbicides
Protein (% in seed)	36.1	38.3	32.2	42.0
Oil (% in seed)	9.1	5.6	5.8	18.0
Lysine	1.58	2.07	1.46	2.56
Cysteine+Cystine*	2.3	3.2	2.0	1.8
Methionine	0.25	0.23	0.19	0.26
Seed Coat (% of seed)	18	25	24	13
Pod Wall (% of Whole Pod)	28	42	32	45
Main Alkaloids	Lupanine, albine***, angustifoline, 13-α-hydroxylupanine, multiflorine, isolupanine	Lupinine, sparteine, gramine***	Lupanine, 13-α hydroxylupanine, angustifoline, isolupanine	Lupanine, 13-α-hydroxylupanine, sparteine

* From Sipsas et al., 2004 using method of Barkkhold and Jensen, 1989.
** Not available.
*** Variously present in some lines.

Source: From Gladstones, 1970; Hove et al., 1978; Culvenor and Petterson, 1986; Röemer and Jahn-Deesbach, 1988; Jones and McLean, 1989; Wink et al., 1995; Petterson et al., 1997; Caligari et al., 2000; Cheng and Jones, 2000; Jones, 2001; Cheng et al., 2002; Jones et al., 2003; Clements et al., 2002, 2004a; Sipsas et al., 2004; Sweetingharr et al., unpublished; Ping Si, personal communication, 2004.

Table 9.8 Major Genes for *Lupinus albus*

Gene	Characteristics
albiflorus (alb)	pure white flowers
Roseus (ros)	pink flowers
pauper (pau)	low alkaloid
brevis (brev)	early flowering, short growth
contractus (con)	early flowering on 1st order branches
Festinus (Fest)	early flowering on branches
ep 1	restricts branching to 1st order (self-completing)
plenus	round seeds
phaseolicus (phas)	round seeds
quadratus III (quad III)	square seeds
quadratus V (quad V)	rectangular seeds
pallidus (pal_1, pal_2)	light green leaves
recessives	dwarfing genes (from XA100)

Source: From Plarre (1991), Gladstones (1970), Pate et al. (1985), Swiecicki (1986b), Harzic and Huyghe (1996).

Sech and Huyghe (1991) reported that heterosis existed for yield in the progeny of a diallel crossing analysis. Whether heterosis can be exploited in this crop would be of interest for further research. Major genes for *L. albus* are listed in Table 9.8. The gene *pauper* for low alkaloid content, selected in the early 1930s in Germany, is now amost exclusively used in breeding programs (Huyghe, 1997), although several other genes are available (Gladstones, 1970; Kurlovich, 2002d). Following the combining of domestication traits such as soft and white seeds, and reduced pod shattering—which were already available from ancient times—with that of reduced alkaloids, there was a focus on increased yield and the reduction of flowering time and excessive indeterminate branching. The new low-alkaloid cultivars were found to have lower yields than comparable bitter lines (Cowling et al., 1998b; Brummund, 2000), and further breeding was required to compensate. The discovery of thermoneutral genotypes (e.g., cv. Neutra, 1959) gave greatly reduced time to maturity and tolerance to delayed sowing in spring (Brummund, 2000). Reduction of plant height in the “short” types by German breeders in cultivars such as cv. Ultra (1950) and Hansa (1954) also facilitated maturity before autumn rains.

Breeding objectives from these times forward, which was carried out mainly by European scientists, depended on whether the crop was autumn and winter or spring sown. The areas for winter and spring sowing have been broadly outlined for the Mediterranean basin by Cubero and López Bellido (1986), based on temperature isotherms. Swiecicki (1986b) described *L. albus* ideotypes suited to these differing European growing seasons based on the available major genes for flowering time and growth habit (*brev*—early flowering, short growth; *Flor*—early flowering linked to *brev; con*—shortens first-order branches; *Fest*—reduces flowering on higher-order branches and linked to *con*). The genotypic combinations were: (1) a spring dwarf type *(brev Flor con Fest)* for northern France, southern U.K., and middle Poland; (2) a typical spring type *(Brev flor con Fest)* for mid- to southern France, Hungary, middle Ukraine, the northwestern U.S., and high rainfall maritime and Mediterranean climates; (3) a late spring type for Mediterranean countries with maritime climates *(Brev flor Con fest);* (4) winter types *(Brev flor Con fest)* for Egypt, Azores, and Canary Islands. Another gene named *ep1* (Mikolajczyk et al., 1984) for the "self-completing" character that restricts branching (determinacy) to the first order was combined with a spring and spring dwarf backgrounds. Determinacy in white lupin is under monogenetic and recessive inheritance (Julier and Huyghe, 1993) and restricts branch orders down to one or two levels and provides earlier maturation in cooler climates (Milford et al., 1993). Winter forms were developed further to incorporate tolerance to low temperature (Wells and Forbes, 1982) and disease resistance, especially brown leaf spot *(Pleiochaeta setosa),* for which recessive resistance from Italy was identified by M.Lenoble in France (Swiecicki, 1986b).

Substantial further development of *L. albus* plant architecture from its classically indeterminate growth habit for various European growing conditions has occurred in the past 15 years (Huyghe, 1991; Milford et al., 1993; Julier and Huyghe,1993; Huyghe, 1997, 1998). This was achieved through the use of determinacy and dwarfism. Several dwarf mutant genotypes have been reported in white lupin (Harzic et al., 1995; Muranyi and Sawicka, 1990; Harzic and Huyghe, 1996). The mutant XA100, artificially induced using cv. Lenoble, and conditioned by two recessive genes, had the benefits of reducing internode lengths on main stem and branches (and therefore reducing lodging) but not changing number of leaves, number of branches, leaf size, and leaf area (Harzic and Huyghe, 1996). Determinate forms of *L. albus* were found to have reduced intra-inflorescence variation in seed size (Crochemore et al., 1994) and higher harvest index than indeterminate types (Schwab et al., 1999). A more-uniform seed size is desirable for processing in some markets, such as in Chile. While grain yield in indeterminate genotypes in maritime temperate environments is not correlated with biomass, it is in restricted branching genotypes. Peel and Galwey (1999) suggested that stable yields for these environments in autumn-sown *L. albus* could be achieved by combining a low vernalization requirement with a long juvenility period, despite the two traits being positively correlated. Based on new understandings of growth, development, and soil adaptation, land suitability maps were developed for autumn-sown genotypes in England and Wales (Siddons et al., 1994). With the development of autumn-sown, dwarf-determinate genotypes with numerous main stem leaves but reduced main stem height, *L. albus* has become a viable crop for cultivation in areas such as northwestern Europe. High-yielding indeterminate cultivars (e.g., cv. Luxe) are, however, also being released in

France and combine frost tolerance with early flowering that prevents excessive vegetative development and, therefore, prevents problems with timely maturity (Harzic et al., 2004). Additional breeding objectives for *L. albus* include reduction of the relatively thick pod wall. Pod wall and seed coat reduction was recommended for *L. albus* originally by Lenoble (1982), and subsequent work has shown that reductions in pod wall proportion are associated with increased yields (Lagunes-Espinoza et al., 1999), heritabilities for the trait are moderate, and that sources of low pod wall can be found particularly in Egyptian germplasm (Lagunes-Espinoza et al., 2000).

Lupinus albus is relatively widely adapted with respect to soil types (Gladstones, 1986). This species produces numerous, well-defined cluster roots, which are induced in response to low phosphorus and iron in the soil (Gardner et al., 1981; Clements et al., 1996; Neumann et al., 1999; Skene, 2003). Cluster roots enable *L. albus* to take up almost five times more phosphorus per unit root length than soybean, which does not form them (Watt and Evans, 2003). In regions of its natural distribution, such as Egypt, *L. albus* is found growing on soils ranging from sand to heavy clays. Germplasm with good tolerance to the abiotic stress induced by calcareous soils have been identified from Egypt (Christiansen et al., 1999; Raza et al., 1999) and from other regions, such as the Bari region, Italy (Huyghe, 1997). Some *L. albus* material from Egypt was found to have tolerance to limed soil at levels found in the tolerant species *L. pilosus* (Kerley et al., 2002). Preliminary results with germplasm have indicated a quantitative inheritance of tolerance to alkaline-induced iron chlorosis (Rogers et al., 2001). Another feature of *L. albus* is that genotypes can accumulate manganese (Gladstones and Drover, 1962).

Frost tolerance is important in autumn-sown cropping systems for *L. albus*. The mechanisms of plant tolerance to frost have been studied and have shown good levels of heritability and of an additive nature (Huyghe, 1997). Sources of cold resistance are likely to be found in germplasm of *L. albus* var. *albus* and var. *graecus* from Italy, Spain, Turkey, and Greece. Despite some evidence for genetic variation for physiological tolerance to drought (Rodrigues et al., 1995), the main avenue of progress in *L. albus* has been achieved through developing the correct phenology, i.e., altering flowering time and plant architecture. Little remobilization of assimilates stored in the stem to seeds occurs in *L. albus* (Withers and Forde, 1979). Development of deeper roots without loss of top biomass and yield may be another characteristic worth exploring in this species. Some germplasm accessions originating from Ethiopia (Francis et al., 1997) with good pod set stayed green for longer under terminal drought conditions, and preliminary observations suggested a stronger rooting system may have been responsible. A more extensive root system and higher leaf area and stomatal conductance gave better drought tolerance in cv. Acores (Rodrigues et al., 1995). Kurlovich and Kartuzova (2002) list cv. Start, K1540, and K2192 as being genotypes with drought tolerance, although this may primarily be due to earliness.

the major fungal diseases affecting *l. albus* are anthracnose *(colletotrichum gloeosporioides),* Pleiochaeta root rot and brown leaf spot *(Pleiochaeta setosa),* rust *(Uromyces lupinicolus),* fusarium wilt (*Fusarium oxysporum* f.sp. *lupini*), and grey mold *(Botrytis cinerea).* Anthracnose was a problem in Central and South America, but it has emerged more widely in the past 15 years in Europe and other countries. Grey mold is more significant in northern Europe, where lupins mature late under very moist conditions. Apart from recessive resistance from Italian germplasm reported by Swiecicki

(1986b), resistance to pleiochaeta root rot has been reported in germplasm from Crete and the Azores (Sweetingham and Yang, 1998). Reference to some levels of tolerance to anthracnose include: von Baer and Hashagen (1999) (cvv. Rumbo, Victoria, and L-7–95); Gondran et al. (1999) (cv. Victoria and GR56); Talhinhas et al. (2000) (cv. Prima Baer); and Adhikari et al. (2003c) (Ethiopian lines P26786, P28523, P28537, and two Portuguese lines). Screening work is in progress with Polish and Russian scientists at Poznan and Bryansk in collaboration with CLIMA (Western Australia) for both anthracnose and fusarium wilt to identify tolerance in *L. albus*, *L. luteus*, and *L. angustifolius*. There is potential to find further sources of resistance to anthracnose in germplasm (e.g., from the Azores and Iberian Peninsula). *Lupinus albus* appears to be resistant to phomopsis, compared to *L. angustifolius*, although there is marked strain specificity (Wood and Allen, 1980; Shankar et al., 1999a; Shankar and Sweetingham, 2001). While *L. albus* is inherently resistant to cucumber mosaic virus (CMV) (Jones and Latham, 1996), it is susceptible to bean yellow mosaic virus, a moderate problem in east and central Europe and the U.S. (Jones and McLean, 1989). The strains that are seed-borne are not present in Australia (Jones, 1997). However, a non-necrotic strain has been identified in Australia, and this is causing some concern (Cheng and Jones, 2000). Sowing seed with minimal BYMV is the most important control strategy.

Current breeding in white lupin is especially focused on improving anthracnose resistance, a disease that limits yields in most countries in which it is currently grown. The albus lupin industry in Western Australia, based on the susceptible Ukrainian variety Kiev Mutant, collapsed as a result of the 1996 anthracnose outbreak. Prior to that, the industry had rapidly expanded to 35,000 ha. There is an opportunity to accelerate a breeding plan to reestablish the albus industry using a source of good resistance from a wild Ethiopian landrace and to develop a pool of elite breeding lines for further yield improvement. A source of resistance was identified in several accessions of landrace material collected in Ethiopia. This resistance was identified using glasshouse resistance screening procedures and has subsequently been confirmed under field disease nursery conditions. The accession P27174 appears to have a level of resistance similar to Tanjil, the most resistant narrow-leafed lupin.

The breeding of dwarf-determinate autumn-sown material is a major advance in the use of *L. albus* in Europe. The determinate growth habit provides earlier and more reliable plant maturity and increases grain yield through reduced competition between vegetative and reproductive sinks environments such as in France and Great Britain. In some environments, such as in Australia and the southern U.S., indeterminate types continue to provide higher yields. Drought-tolerant types and improved partitioning of assimilates to pods can be bred by using early flowering with less main stem leaves but with several primary branches to maximize light interception and yield potential. Programs must also be concerned with the maintenance of high protein and oil, while improving yield. Some variation for protein and oil has been reported in germplasm collections (Kurlovich and Kartuzova, 2002). Apart from the use of the Dragendorf reagent test for rapid detection and elimination of bitter types from segregating populations and cultivars grown in near proximity to bitter types, UV fluorescence of seeds is also being employed as a rapid and time-saving screening method (von Baer and Perez, 1991). Breeding programs in Europe will still require the incorporation of a good source of anthracnose resistance if the crop is to achieve popularity.

9.5.1.2 Lupinus luteus

Outcrossing rates indicate that yellow lupin is both self-compatible and posseses the ability to outcross to moderate levels. A maximum of 8% outcrossing was recorded between rows spaced as close as 0.25 m, and outcrossing rates decreased rapidly within 6 m and fell to nil after 25 m from the pollen source. (Adhikari et al., 2003a). Wallace et al. (1954) found outcrossing rates for *L. luteus* to vary from 2.6 to 8.2% across two years for rows spaced 1 m apart. For mingled plants, however, they found outcrossing rates of up to 40%. The possible existence of heterosis and inbreeding depression in *L. luteus* warrants further investigation. In more highly outcrossing New World annual species such as *L. texensis*, overall inbreeding depression can be as high as delta= 0.66, which is more than twice the level required for maintenance of an outcrossing mating system (Helenurm and Schaal, 1996). Some of our observations suggest that inbreeding depression may be occurring in breeding programs for yellow lupin.

The main centers of yellow lupin breeding have been Germany, Poland, Belorussia, Ukraine, and Russia. Low alkaloid genes reduced the levels of the major alkaloids of yellow lupin, sparteine, and lupinine, from their levels of 1 to 4% in seed and 0.2 to 0.5% in shoot dry matter (Table 9.9) (Hackbarth and Troll, 1956). Levels of from 0.08 to 0.1%, as in cv. Teo, to as low as 0.002%, as in cv. Wodjil in Western Australia (Cowling and Gladstones, 2000), have been achieved using the original natural mutant genes for low alkaloid. Other minor alkaloids, such as gramine, which were

Table 9.9 Major Genes for *Lupinus luteus*

Gene	Characteristics
dulcis (dul)	low alkaloid
amoenus	low alkaloid
w	permeable seeds
alb	white seed coat
coloratus niveus (col^{niv})	white seed coat, lower anthocyanin, lighter shoots
coloratus falcatus (col^{falc})	white seeds, eyebrow, dark spots
coloratus parvimaculatus (col^{Parv})	uniformly spotted seed coat
invulnerabilis (inv)	nonshattering pods
Coloratus (Col)	black seed coat
Fuscus (Fusc)	greyish-black seed coat
fuscus (fusc)	brown seed coat
sulfureus (sulf)	sulfur-yellow flowers
flavus (flav)	dark yellow flowers
Rufus (Ruf)	“orange” flowers
helvis (hel)	amber flowers

crescens celer ($cres^{cel}$)	rapid early growth, lighter leaves
crescens altus ($cres^{alt}$)	very rapid early growth, lighter leaves
promptus (prompt)	rapid early growth, normal leaf color
Rapidus (Rp)	rapid early growth
alt	tall stem
nanus	dwarf plant structure
olivaceus (oliv)	dark green leaves
aureus (aur)	light green leaves
dilutus (dil)	light green leaves, stems
Fusariosus (Fus)	fusarium wilt resistance
Erysiphus (Er)	powdery mildew resistance
Ncm-1	hypersensitivity-type resistance to CMV

Source: From Gladstones (1970), Pate et al. (1985); Swiecicki (1986b); Jones and Latham (1996); Swiecicki and Swiecicki (2000); Kurlovich (2002d).

only discovered after the development of improved methods of alkaloid analysis, occured in all *L. luteus* wild and landrace germplasm. This alkaloid was found to have a negative influence on fodder palatability. A progressive decline in the presence of gramine apparently occurred in breeding material in Poland, although higher amounts have still been found in some lines. For example, in cv. Teo, approximately 90% of the alkaloids are gramine (Swiecicki and Swiecicki, 2000). Three low-alkaloid genes were identified in yellow lupin (*amoenus, liber,* and *dulcis*), one of which was associated with poor vigor *(liber).* The gene *dulcis,* developed by von Sengbusch, appears to have been used more frequently in breeding programs (Gladstones, 1970; Pate et al., 1985). Reduced pod shattering was provided through the gene *invulnerabilis* (Hackbarth, 1957a), which provided large yield increases in European crops of yellow lupins. In Mediterranean environments with dry finishing conditions, pod shattering still occurs, and there is a need for additional or alternative genes.

After initial domestication by combining a number of recessive alleles (including *w* for permeable seeds), further improvements in yellow lupin were achieved by creating more rapidly growing and earlier flowering genotypes with higher yield, both for green and dry fodder and for seed (Swiecicki and Swiecicki, 2000). This was achieved through the identification of genes such as *crescens celer (crescel)* in cv. Weiko III and *crescens altus (cresalt)* in cv. Alteria. Both these genes are pleiotropic and give a lighter green foliage color (Hackbarth and Troll, 1957a). Another gene, *promptus (prompt),* accelerates growth but does not affect leaf color (cv. Expires). Additionally, the gene *Rapidus (Rp),* from the Netherlands, gives rapid early growth and had a pleiotropic effect on dark green leaf color, and gave a stronger main stem base (cvv. Palvo, Juno, Teo). A mutant gene for tall stem development *(alt)* was also identified. A problem did arise, however, with the overreliance on a number of these mainly recessive genes. Lines carrying the genes *cres* and *alt* together were found to have reduced root growth and, therefore, yield reductions

under certain environments (Brummund, 2000). The saturation of breeding material with the recessive alleles in the period from 1940 to the 1970s in several cultivars, such as Refusanova, resulted in lower yields relative to bitter types or those carrying few recessive genes. Cultivars after that period, which contained fewer of these genes, were found to recapture higher yield levels (Brummund, 2000; Swiecicki and Swiecicki, 2000). One of the genes dispensed with was the *alb* gene for white seed coat, which was no longer seen as being necessary in German programs.

Plant architecture is typically characterized by an early rosette stage followed by indeterminate branching. The gene *Therm* provided thermoneutrality and, therefore, removed vernalization requirement, resulting in earlier flowering even when crops were sown in late spring (Swiecicki and Swiecicki, 2000). The gene also provided escape from viral infection. Examples of genotypes carrying the *Therm* gene are Juno, Borsaja, and Teo. A gene for restricted branching, *rb*, was discovered in Hungary and introduced into a range of cultivars such as Manru, Borselfa, Radames, Markiz, and Legat (Swiecicki and Swiecicki, 2000). Genotypes with this gene were referred to as "self-completing" yellow lupins, and they provided advantages similar to analagous types in *L. albus*. Swiecicki and Swiecicki (2000) found restricted branching cultivars to be highly suited to higher rainfall but shorter growing season environments. Another gene, *bbr*, for absence of basal branching is reported by Kurlovich (2002d).

Yellow lupin has some susceptibility to frost, particularly in the earlier growth stages but also when racemes are exposed to transient frosts during flowering and podding. Germplasm is not generally found at altitudes of much higher than 500 m. Further evaluation and collection of germplasm from higher altitudes may offer some source of frost resistance. Interspecific crossing with *L. hispanicus* ssp. *hispanicus* and ssp. *bicolor* could offer resistance to low winter temperatures (to −15C) (Swiecicki, 1986a), important for inland Spain and for extending the crop into northern Europe. Yellow lupins are tolerant of soil aluminum (French et al., 2001) and are best suited to soils from pH 4.5 to 6.5. Breeding *L. luteus* to tolerate higher soil pH may be possible through the use of germplasm that collected from higher pH sites. Some accessions held in the ALC were collected on soils with a pH ranging from 7 to 8.5 by Francis in Morocco. While *L. luteus* is more efficient at taking up soil phosphorus, it takes up cadmium, often to above maximum permissible levels (Brennan and Bolland, 2003). A possible reason for this efficient uptake of nutrients is the ability of *L. luteus* to produce a large number of third-order lateral roots with a structure not dissimilar to proteiod roots (Brennan and Bolland 2003). Screening for variation among breeding lines or germplasm for uptake of cadmium may be worthwhile. *Lupinus luteus* has been found to be relatively waterlogging tolerant, a trait of some importance in the wetter months of autumn-sown crops in Mediterranean environments (Davies et al., 2000). Little information is available on the status of *L. luteus* in relation to drought tolerance. *Lupinus luteus* accumulates less ABA in roots, relative to other lupin species (Hartung and Turner, 1998) and is considered to be drought susceptible (compared with *L. angustifolius*) in Mediterranean climates, and the species is better suited to higher rainfall cropping zones (Gladstones, 1994). Swiecicki and Swiecicki (2000) suggest that genotypes with a higher concentration of anthocyanin and chlorophyll (through the gene *oliv* for leaf color) but with short growing periods are more resistant to drought than types having genes for fast growth and with the light green leaf gene *au*. Another recessive gene responsible for light

green leaf color, *dilutus*, was also found to reduce fertility (Kazimierska and Kazimierski, 1995).

Very low alkaloid *L. luteus* genotypes such as cv. Wodjil can be severely damaged by aphids (Berlandier and Sweetingham, 2003). Efforts are under way to determine mechanisms of tolerance and breeding for tolerance by maintaining alkaloid levels at 0.02% or below but by manipulating alkaloid profile (Adhikari et al., 2003b; Edwards et al., 2003). CMV is a serious problem in east and central Europe where it is carried over through lupin seed and causes 'lupin browning disease'. Resistance to both plant infection and to seed transmission is available. Resistance to seed transmission is not related to alkaloid content or flowering time and is probably polygenically controlled (Sweetingham et al., 1998). A single dominant hypersensitivity gene, *Ncm-1*, has been identified, which is responsible for resistance to CMV in cv. Wodjil and other genotypes (Jones and Latham, 1996). BYMV is a serious viral disease in east and central Europe, where it reduces yield and is often called 'lupin narrow leaf virus'. It also occurs in the U.S. The virus is readily carried over through lupin seed. Partial and quantitative resistance to BYMV has been used in breeding in Europe, particularly through the use of thermoneutral types, which are less likely to become infected (Jones and McLean. 1989; Sweetingham et al.. 1998; Swiecicki and Swiecicki, 2000). Virus diseases in Western Australia are generally restricted to areas with greater than 350 mm annual rainfall.

Fungal diseases of yellow lupin include anthracnose, fusarium wilt, and powdery mildew. Cultivars have moderate resistance to phomopsis (Shankar et al., 1999a, 1999b). Fusarium wilt has caused large yield losses in the past in Europe and out of three races, races 1 and 2 were the most pathogenic on *L. luteus* (Sweetingham et al., 1998). Resistance was first identified in Portuguese germplasm, and later found in other populations from Sicily (Swiecicki and Swiecicki, 2000). The gene *Fus1* was then transferred to cultivars such as Refusanova, Borluta, and Bornova (Germany), and Afus and succeeding Polish cultivars. The resistance provided by this gene has been remarkably stable over time (Swiecicki and Swiecicki, 2000). Resistance to powdery mildew was provided firstly by Portuguese germplasm, then by the gene *Er*, which was derived from Spanish germplasm (Lamberts, 1955; Gladstones, 1970). Although crop rotation, fungicide seed dressing, and use of clean seed is providing control, screening for anthracnose resistance in yellow lupin is currently being conducted in Poland and Russia. There has been a report of sources of resistance from Portuguese lines (Weimer, 1952; Sweetingham, 2000) and some suggestion that cvv. Cardiya and Estoria and breeding lines from Bryansk have moderate resistance.

Experience with *L. luteus* in Western Australia up until the mid-1970s was typified by problems with drought-susceptibility, aphid and budworm attacks, BYMV seed infection, low harvest index and yield, and small seed size (Gladstones, 1994). The gene *cresens celer* and *Rapidus*, for erect and early vigor, were associated with lodging, and the genes probably gave a lower yield potential under those climatic conditions. *L. luteus* breeding in Australia was not taken further until the 1990s when limited efforts produced cv. Wodjil, a reselection from cv. Teo. Further breeding of *L. luteus* in Australia will concentrate on anthracnose resistance, aphid tolerance, reduced pod shattering, and breeding for higher harvest index and yield accompanied by lodging resistance. Exploration of different cominations of the various genes for earliness, vigor and plant architecture may provide improvements. Reduction of pod wall proportion, which is

unusually high in this species, may also provide yield increases. Seed coat proportion of total seed weight is also relatively high (Clements et al., 2002; Clements et al., 2004a), and some reduction could be achieved through increases in seed size (use of larger seeded 'ssp. *orientalis*' types) or through identification of thinner seed coat in germplasm or mutation populations. Yield improvements of approximately 30% over cv. Piast (registered in 1987) have been achieved in Poland in recent times, with newer culivars such as Polo (1997) and Lidar (1998) (Swiecicki and Wiatr, 2001). The major direction in breeding during this period has been concerned with restricted branching and thermoneutrality, and future aims include breeding for anthracnose resistance and achieving consistently low alkaloid content in seed.

A premium for yellow lupin grain is projected in Australia because of its greater protein and sulfur amino acid content (Table 9.7) (Petterson et al., 1997; Sipsas et al., 2004). A better assessment of available variation in breeding lines and germplasm for protein and sulfur amino acid content in *L. luteus* may be possible with improved analytical methods (Sipsas, 2003; Sipsas et al., 2004) and rapid screening techniques, such as near infrared spectroscopy (Pazdernik et al., 1997). Potential for selecting higher.protein content in germplasm may be possible, based on the data of Gladstones and Crosbie (1979). Introgression of traits from *L. hispanicus*, such as protein content, walterlogging tolerance (particularly *L. hispanicus* ssp. *bicolor*), yield (*L. hispanicus* ssp. *hispanicus*), and brown leaf spot [*L. hispanicus* ssp. *bicolor* from Turkey; Wells, and Forbes (1982)] might be possible.

9.5.1.3 Lupinus angustifolius

Lupinus angustifolius is regarded as a self-pollinated species, with automatic self-pollination occurring usually before the petals open (Free, 1993). Outcrossing rates have been reported to range from practically zero (Wallace et al., 1954), from 0 to 2% (Quinlivin, 1974; Dracup and Thomson, 2000b), and from 0 to 12% (Forbes et al., 1971), with the latter reporting variability due to cultivar, season, site, wind, and presence of pollinators. Outcrossing has been attributed mainly to insects, particularly honeybees, which can force the keel to protrude through the wing petals and allow pollen transfer (Leuck et al., 1968; Langridge and Goodman, 1977). Pollen of *L. angustifolius* is too large and sticky to be carried any significant distance by wind (Langridge and Goodman, 1977). Restricted branching lupins have shown a characteristic of precocious and abnormal flower opening of flowers in the leaf axils (Dracup and Thomson, 2000a). Dracup and Thomson (2000b) found; however, that outcrossing rate in *L. angustifolius* was not affected by plant architecture (normal vs. restricted branching) or flower position on the mother plant.

Narrow-leafed lupin breeding began in Germany, Poland, and also in Russia, with the discovery of low-alkaloid natural mutants (Gladstones, 1970, 1998; Swiecicki and Swiecicki, 1995; Kurlovich, 2002a) and was followed by the intensive work in Australia beginning in the late 1950s and some parallel and collaborative work in the U.S. (Forbes and Wells, 1966; Gladstones, 1994, 1998; Cowling et al., 1998a, 1998b; Cowling and Gladstones, 2000). The crop has played a very important role in Australia since the early 1980s, in rotation particularly with cereals and as a feed for sheep, pigs, and poultry. It is

also grown currently on a commercial scale in Poland, Byelorussia, the Baltic countries, Russia, and South Africa.

Lines of *L. angustifolius* evaluated in the 1950s in Australia were initially very late flowering and not productive in the environments in which they were first tested. *L. albus* was not adapted to the mostly infertile sands in the Western Australian wheatbelt, and *L. luteus* cv. Weiko III was found to be drought sensitive and prone to virus and insect attack. The crop improvement program based in Perth by J.S.Gladstones then focussed efforts on *L. angustifolius*. Genes that were found as naturally occurring mutants in cv. New Zealand Blue for nonshattering pods (*le* and *ta*) and white flowers and seeds *(leuc)* were combined with the naturally occurring mutant gene for early flowering and thermoneutrality *(Ku)*, along with low alkaloid (*iuc* from cv. Borre), and permeable

Table 9.10 Genes Reported for *Lupinus angustifolius*

Gene	Characteristics
retardans (ret)	large seeds and fast growth
procerus (proc)	tall, fast growth
properans (prop), lat	rapid growth, tall, less branching, epistatic to proc
mollis (moll)	permeable seed coat
leucospermus (leuc)	white flowers and seeds
albus (alb)	whitish flowers and seeds
albiflorus (as)	white-yellow flowers, beige seed
roseus (ros)	pink flowers
subcoeruleus (scoer)	blue-grey flower color
subalbidus (salb)	white-blue flowers
discolor (dis), dispersus (dip)	lighter blue flower color
Supercoeruleus (Sup)	intense blue flower color
iucundus (iuc)	low alkaloid
esculentus (es)	intermediately low alkaloid
depressus (depr)	very low alkaloid
tantalus	low alkaloid
tardus (ta)	reduced shattering pods
lentus (le)	reduced shattering pods
efl	reduced vernalization requirement, earlier flowering
Ku	no vernalization requirement, early flowering
Linifolius (Lin)	narrow and short leaflets

latifolius (lat)	broad and long leaflets, rapid growth, possibly=prop
purpureus (pur)	high anthocyanin in leaves
viridus (vir)	light green leaves
aurifolius (af), virescens (vires)	very light green leaves
mut-1	highly restricted branching=rb3
Det	restricted branching=Rb1
An	anthracnose resistance
Anr1	anthracnose resistance
gl1	grey leaf spot resistance
gl2	grey leaf spot resistance
Phr1	phomopsis resistance
Phr2	phomopsis resistance
Rfo1	fusarium resistance
Rfo2	fusarium resistance

Source: From Gladstones (1970), Pate et al. (1985); Swiecicki and Swiecicki (1995); Kurlovich (2002d); Kuptsov et al. (2004d).

seed *(moll)* genes (Table 9.10). A series of cultivars beginning with cv. Uniwhite in 1967 *(moll, iuc, leuc, ta),* Uniharvest, 1971 *(moll, iuc, leuc, to, le),* and Unicrop, 1973 *(moll, iuc, leuc, ta, le, Ku),* were the beginning of the highly successful history of narrow-leafed lupin in Australia (Gladstones, 1994; Cowling and Gladstones, 2000). The program relied upon white flowers and seed as markers and the strict removal of bitter plants to ensure quality and purity in cultivars. Alkaloid levels in Australia are now governed by food and feed standards, which specify an upper limit of 0.02% total alkaloids in whole seeds for varieties (Culvenor and Petterson, 1986). This has allowed the use of the commercial term Australian Sweet Lupins for *L. angustifolius* seed exported from Australia. Similar to the European experience with yellow lupins, a negative effect of domestication genes on yield and other characters occurred in *L. angustifolius.* The *iuc* gene for low alkaloid and, to some extent, the *ta* gene for nonshattering, had negative pleiotropic effects on the severity of the split-seed disorder caused by manganese deficiency on infertile sandy soils (Walton and Francis, 1975). Isogenic lines of earlier Australian *L. angustifolius* breeding material with the *iuc* gene were 30% lower yielding than bitter lines (Oram, 1983). It is not surprising, with these kinds of results for *L. angustifolius* and with the general observation that low alkaloid lupin crop genotypes have both greater pest susceptibility and possibly lower inherent yield potential, that the concept of the 'bitter-sweet' plant ideotype has arisen. Lupin seeds do not produce alkaloids but store them (Wink, 1994). The 'bitter-sweet' concept proposes the generation of a plant with high alkaloids in vegetative tissues, but which does not translocate alkaloids to seeds possibly through the manipulation of alkaloid transporters (Wink, 1991, 1994). Although the gene *le* for reduced pod shattering through modification of the pod endocarp (Gladstones, 1967) had

some negative effects on plant growth, the gene *ta* for reduced shattering had positive effects on plant growth and yield (Cowling et al., 1998b). Breeding over the past 20 years has improved yield to compensate for this apparent physiological deleterious effect of the gene for lower alkaloid content, which is in addition to the effect of greater general susceptibility to insects and, in some cases, diseases.

Following the initial phase of domestication, the next phases of breeding *L. angustifolius* in Australia were the incorporation of disease resistance genes from wild germplasm and selection for yield improvement through both pedigree selection and recurrent selection breeding methods (Gladstones, 1994; Cowling and Gladstones, 2000). Use of F_1s in crosses back to elite breeding lines was also common in the Australian program since the 1980s. Traits incorporated included grey leaf spot *(gl1)* and anthracnose *(An)* resistance from U.S. cv. Rancher, phomopsis resistance from Spanish and Moroccan wild types, and brown leaf spot and Pleiochaeta root rot resistance from Israeli and Italian wild types (Cowling and Gladstones, 2000). With the outbreak of anthracnose in 1996 in Australia, intensive efforts were made to screen existing cultivars and germplasm for resistance, both within Australia and offshore (Cowling et al., 2000). The preemptive intermediate resistance, possibly originating from American breeders (*An* gene) (Gladstones, 1970), that was available in cvv. Illyarrie and Yandee, and stronger resistance from cvv. Tanjil and Wonga (*Anr1* gene) (Yang et al., 2004), has meant that the disease is a lower threat in *L. angustifolius*. Another separate moderate resistance exists in cv. Kalya and Mandelup (Yang and Buirchell, unpublished, 2004). Improved resistance to anthracnose infection of pods and alternative stem resistance is still a breeding aim.

Considerable efforts are now being carried out to assist in the characterization of the fungus (Talhinhas et al., 2002). There is still some confusion over whether the pathogen should be called *Colletotrichum gloeosporiodes* or *C. acutatum*. Nirenberg et al. (2002) provided evidence that the lupin pathogen is unique enough to consider erecting a new species and has proposed the name *C. lupini*. Yang and Sweetingham (1998) examined 160 isolates of *Colletotrichum* from lupin worldwide and found they fell into three vegetative compatibility groups (VCGs). Each VCG has a distinct RAPD-PCR profile. VCG 2 is by far the most common, having been identified from Australia, Chile, Germany, Poland, Portugal, the U.S., and the UK. VCG 1 was only detected in France and Canada. VCG 3, although isolated from lupin, appeared primarily a pathogen of strawberry. Research by Nirenberg et al. (2002) and Talhinas et al. (2002) supports the existence of two main strains of the anthracnose pathogen. Both strains, however, appear to have similar pathogenicity and host preference.

The most important fungal disease in *L. angustifolius* currently in Europe is fusarium wilt, and resistance is available in programs based there. The races of the fusarium wilt pathogen in Germany, Poland, Ukraine, Belarus, and Russia vary in their specificity among *L. angustifolius, L. albus,* and *L. luteus* (Lamberts, 1955; Armstrong and Armstrong, 1964; Furgal-Wegrzycka, 1984). Local breeding programs have bred for resistance in each region, but resistant cultivars do not always show resistance elsewhere. For example, the Australian cultivar Tanjil has been reported to be resistant in Belarus (Kuptsov et al., 2004b) but is very susceptible in Poland (Sweetingham and Frencel, unpublished, 2004). *Pleiochaeta setosa* causes brown leaf spot in *L. angustifolius* but can be controlled by minimum tillage, stubble retention, and fungicide seed dressings.

Recurrent selection in Western Australia produced cv. Myallie with improved resistance (reduced defoliation), which is polygenic in nature (Cowling et al., 1997). The mycotoxin of Phomopsis *(Diaporthe toxica)* has caused lupinosis in sheep in the past, usually without major effects on seed yield (Cowling et al., 1987; Cowling and Wood, 1989; Williamson et al., 1994). A dominant *(Phr1)* resistance gene originating from the breeding line 75A258 (Moroccan wild type parent), and an incompletely dominant gene *(Phr2)* for resistance in cv. Merrit (Spanish parent), have been developed in the Australian breeding program (Shankar et al., 2002). Two DNA markers are available for *Phr1* (Yang et al., 2001, see Molecular Markers section below).

CMV is a serious problem in *L. angustifolius*. The species has high susceptibility, particularly in late flowering cultivars. The virus is seed borne with partial resistance to seed transmission used in breeding in Western Australia and is now present in some local cultivars. Resistance is probably polygenically controlled and not related to plant alkaloid content (Jones and McLean, 1989; Jones and Cowling, 1995; Jones, 2001). BYMV is also a serious viral disease in *L. angustifolius*, which has high plant susceptibility with seed transmission depending on isolate. Western Australian isolates are not seed borne, but seed transmission occurs in other countries. Necrotic and non-necrotic strains of BYMV occur, with the necrotic reaction being a form of systemic hypersensitivity. Resistance to infection by aphids occurs (Jones and McLean, 1989; Sweetingham et al., 1998; Cheng and Jones, 2000; Cheng et al., 2002; Jones, 2001; Jones et al., 2003).

Yield improvements in *L. angustifolius* have been through a combination of phenological improvements and disease resistance. In wild and landrace germplasm lacking any early flowering genes, time to flowering appears to be influenced primarily by vernalization, with only minor influence of photoperiod (Rahman and Gladstones, 1972; Rahman and Gladstones, 1974). Landers (1995) showed that this vernalization response can range from absolute (essential for flowering), to a reduced response (vernalization not essential as provided by the *efl* gene), to no response in genotypes carrying the *Ku* gene for early flowering. In these early flowering genotypes, flowering time was determined by the combined effects of average temperature and average daylength between sowing and flowering (Reader et al., 1995). High-yielding cultivars in Western Australia carry the *Ku* gene and are predominantly of indeterminate branching architecture. Yield increases of approximately 3% per year have been achieved from the first cultivar release in Western Australia in 1967 to the present (Figure 9.5a). There have been progressive increases in harvest index (Tapscott et al., 1994) from approximately 0.24 in cv. Unicrop to 0.29 in cv. Merrit and further to approximately 0.34 in the latest cultivars (Figure 9.5b). Comparable figures of both harvest index and water use efficiency show that lupins still fall below that of some other legumes, such as *Vicia faba* and *Pisum sativum* (Siddique et al., 2001). *Lupinus angustifolius*, while efficient at remobilizing nitrogen from vegetative structures, does not remobilize carbon well into seed (Pate et al., 1998). Pre-anthesis assimilates are used mainly for stem and pod development rather than for filling seeds, even under drought stress, and there has been no evidence of variation among genotypes (Palta, personal communication). The changes in harvest index in historical Australian cultivars has been associated with improved pod set and yield at higher plant densities (Tapscott et al., 1994), decreases in plant height (Figure 9.5c), and more recent reductions in upper primary branch length since cultivars such as cv. Danja (1986) with long branches (data not shown). Drought tolerance is

achieved through earlier flowering and, in more recent cultivars, rapid pod set and seed growth (Dracup et al., 1998; Palta et al., 2003, 2004).

As with the other crop lupin species (except *L. mutabilis*), *L. angustifolius* has thick seed coats, high dry matter in pod walls, and thick kernel cell walls (Brillouet and Riochet, 1983; Clements et al., 2002). The proportion of seed hull is about 24% in *L. angustifolius* domesticated lines (Lush and Evans, 1980; Miao et al., 2001; Clements et al., 2002) compared with approximately 7% in soybean and 9% in field pea. Dehulling lupins improves nutritive value for monogastric animals such as pigs, poultry and fish species (Edwards and Barneveld, 1998). In current varieties of *L. angustifolius*, about 32% of the pod dry matter is in the pod walls (Dracup et al., 1998; Clements et al., 2002). Reducing seed coat can potentially increase protein or protein plus oil percentage in seed (Clements et al., 2002), and lowering pod wall could improve yield in this species as has been indicated for *L. albus* (Lagunes-Espinoza et al., 1999). Development of rapid screening methods for seed coat proportion and thickness will facilitate breeding (Clements et al., 2004b). Heritability for seed coat and pod wall proportion is moderate or moderate to high (Clements et al., 2002; Mera et al., 2004), and reasonable variation for these traits can be found in germplasm (Clements et al., 2004a).

The cell walls in *L. angustifolius* kernels are composed of nonstarch polysaccharides, (NSP) and form approximately 23% of seed weight. Although this NSP is a valuable dietary fiber with cholesterol-lowering properties (Evans, 1994), it can reduce digestible energy in monogastric diets. It may be possible to decrease NSPs, either through direct breeding and selection or indirectly through selection for higher protein. In soybean, a strong negative correlation has been reported for seed cell wall polysaccharides and protein plus oil concentration (Stombaugh et al., 2000). Brillouet and Riochet (1983) also found a strong negative correlation between protein plus oil concentration and percentage cell wall material in kernels in various lupin species. A relatively narrow range of oil contents occurs in germplasm of *L. angustifolius* (Gladstones and Crosbie, 1979). Some relationship was identified for protein content in seeds and collection site (Gladstones and Crosbie, 1979). A relatively higher proportion of accessions with higher protein contents tended to originate from Morocco and lower contents from Spain and Portugal with eastern Mediterranean accessions intermediate. A recently produced breeding line in Western Australia, WALAN2173M, has consistently shown a 2% higher seed protein than comparable cultivars (Sweetingham et al., 2004). Breeding lines with several percent higher protein than average have also been reported by Ageeva (2004), Hauksdóttir et al. (2004), and Kurlovich and Kartuzova (2002).

Sources of branching modification have been available in *L. angustifolius*, both as naturally occurring and induced mutants, and detailed studies of their botany are available (Dracup and Kirby, 1996; Dracup and Thomson, 2000a, 2000b). Polish and Russian breeding programs currently use restricted branching to provide more reliable yield and maturity for spring-sown crops (Swiecicki and Wiatr, 2001; Kurlovich and Kartuzova, 2002). Dominant and recessive genes controlling resticted branching in *L. angustifolius* are reported and include mut-1 (referred to as a “self-completion” type) (Bromberek et al., 1984), Deter (Kurlovich, 1986), Det (from I.Forbes in the U.S.) (Gladstones, 1994). In attempting to characterize a number of different sources of

restricted branching in *L. angustifolius,* Adhikari et al. (2001) renamed mut-1 and Det as *rb3* and the incompletely dominant Rb, respecitively. Another incompletely dominant

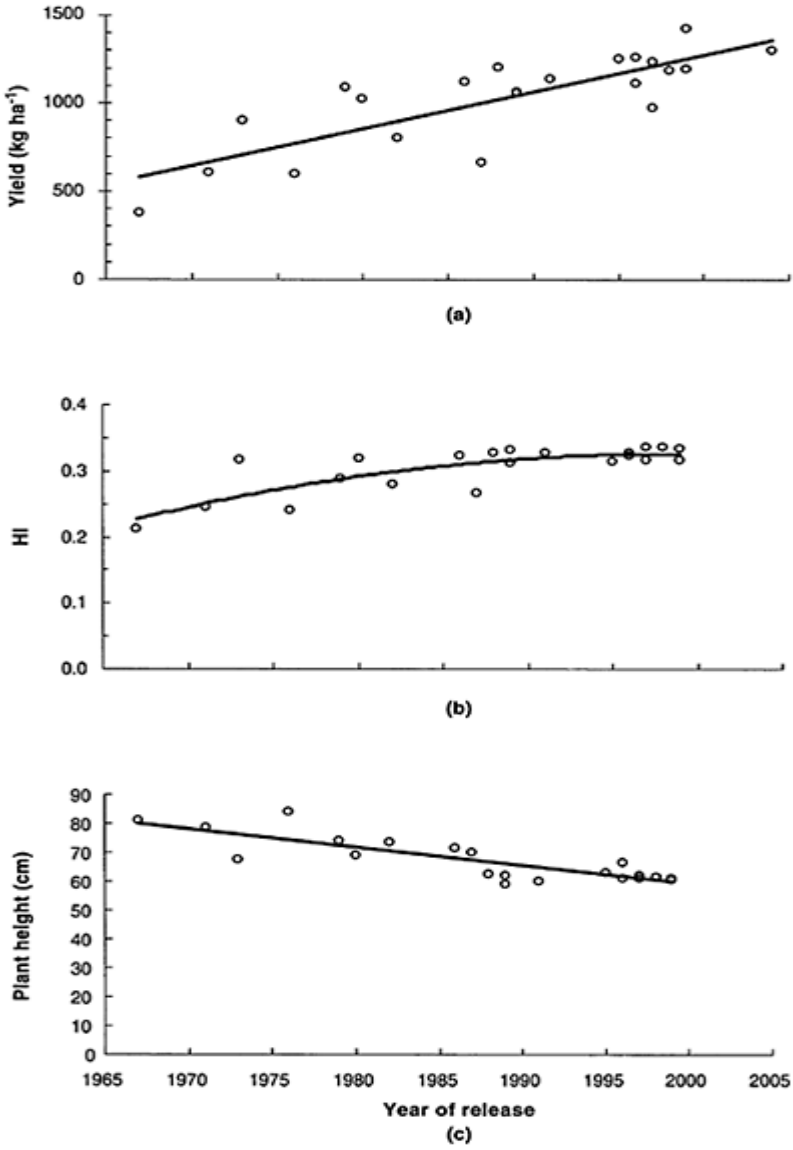

Figure 9.5 Historical *Lupinus angustifolius* cultivars released from the Department of Agriculture Western Australia since 1967: (a) yield

improvements, (b) changes in harvest index, and (c) plant height.

gene, *Rb2*, was also described. Mildly restricted branching in cultivars such as cv. Tallerack was found to be quantitative but highly heritable and controlled by additive genetic effects (Adhikari et al., 2002). Kuptsov (2000) categorized seven plant architectures in *L. angustifolius* and proposed several recessive alleles. The indeterminate structures were divided into the fully indeterminate "wild" branching structure and the "pseudo-wild" type with reduced lateral branch length and fewer orders of branching. Leaf traits associated with the wild growth habit included xeromorphic morphology and dark green leaf color (Kuptsov et al., 2004a). The remaining categories were characterized by restricted branching to varying degrees ranging from mild ("quazi-wild") to extreme ("ear") with no branches and a "palm-like" form with its faciated stem and cluster of pods on a terminal raceme types. Generally, yields from restricted branching genotypes of *L. angustifolius* have been lower than indeterminate types because of their lower leaf area and duration, susceptibility to leaf defoliating diseases, and poorer weed competition. In maritime climates, yields of restricted branching types were found to be approximately 20% lower than indeterminate types (Joernsgaard et al., 2004). Yield advantages occur in some cases for breeding lines with either highly restricted or mildly restricted plant type for specific years and locations. Galwey et al. (2003) incorporated different sources of the restricted branching trait into a range of backgrounds through backcrossing and showed that there were no consistent differences in performance of mild compared to highly restricted branching types and that frequent yield advantages occurred over normal branching types, particularly in a high rainfall, long growing season site in Western Australia. In lines with restricted branching, results indicated that a larger number of leaf nodes on the main stem conferred a yield advantage. The results supported the further development of the trait for use in Australian breeding programs.

Current breeding aims in *L. angustifolius* include improved anthracnose resistance, maintenance of phomopsis and pleiochaeta resistances, reduced seed coat and pod wall proportion, improved herbicide tolerance (e.g., to metribuzin), increasing protein content and sulfur amino acid levels. Further development of both restricted branching and indeterminate cultivars is occurring in Europe, along with a focus on anthracnose and fusarium resistance, cold tolerance, and maintaining low alkaloid levels. Breeders in Western Australia are currently using a form of the recurrent introgressive population enrichment (RIPE) breeding system, which maintains a high level of elite genetic material while progressively introducing new gene and gene complexes via new germplasm (Kannenberg and Falk, 1995). This will facilitate a widening of the superior, but relatively narrow, genetic base of what has been established in the series of 20 or so cultivars registered by the Department of Agriculture, Western Australia, but without breaking up the high yield genetic backgrounds in the latest releases.

9.5.1.4 Rough-Seeded Lupins

The level of outcrossing was determined in a wild population of *L. pilosus* in Israel as between 30 and 60%, depending on the environment (Horovitz and Harding, 1983). It

was able to produce seed at reasonable levels in the absence of pollinators, indicating a versatile mating system of selfing and outcrossing. Reports on the levels of outcrossing in the other related species have not been found. The domestication and development of several of the rough-seeded lupin species as crop plants has occurred primarily in Western Australia through a combination of interspecific crossing and mutation breeding (Buirchell and Cowling, 1992). Interest in the group started because of the existence of naturalized populations of *L. cosentinii* on sandy coastal soils of Western Australia, and this was the first species to be domesticated (Gladstones, 1982). The rough-seeded lupins provide a unique set of adaptations. *Lupinus pilosus* is adapted to fine-textured, neutral to alkaline soils (Tang et al., 1995), and both *L. pilosus* and *L. atlanticus* show good tolerance of free lime (Brand et al., 2002). Seed protein contents range from 26% in *L. pilosus* to 32% in *L. cosentinii* (Petterson et al., 1997). The following described breeding work conducted in Western Australia.

9.5.1.5 Lupinus atlanticus

Over a number of years, different accessions have been tested for yield at a number of sites within Western Australia. There was a large variation in the final yield of accessions from all geographic origins of accessions. Accessions from the northern region of the Atlas Mountains were poorer yielding than accessions from other regions. The yield results seem consistent with the results from the phenotypic analysis indicating that the northern population is significantly different from the rest of the populations, and that there is great variability between accessions within the same region. Interestingly enough, the line from the valley region was considerably better at a more southerly, higher-rainfall site in Western Australia, emphasizing that these accessions are probably well adapted to colder climates. In all the trials, the highest yielding accessions came from the central region.

Mutation breeding was used to enhance the diversity in *L. atlanticus* and to produce characters that are not naturally available. These characters can include many of the domestication characters. Most of the mutations have come about from treatment with sodium azide (Az), ethyl methyl sulphonate (EMS), or radiation. Several mutant genotypes produced include lines with low alkaloids (3 plants), reduced shattering pods (2 plants), water permeable seeds (1 plant), lack of vernalization (1 plant), and white flowers and white seeds. Low-alkaloid lines have been produced in *L. atlanticus* with EMS. There are three single recessive genes for low alkaloids in AM2.8.2 *(sw1)*, AM3.6.1 *(sw2)* and AM4.8 out of another program in 1994. All three lines have alkaloid levels below 0.03%, compared to the natural level of 0.25 to 0.50% dry weight. Two distinct characters for pod with resistance to shattering have been produced. The characters are similar to those discovered in natural populations of *L. cosentinii*. The first has the dorsal seam of the pod welded together, thus stopping the pod from splitting apart at the top (AM5.1). The second (AM4.33) has lost the inner pod lining, which, although allowing the pod to open, drops its seed rather than flinging them out of the pod. Both these single recessive genes are complementary to one another and together give full shattering resistance. The water permeable mutant (AM5.2) was an unstable character that reverted back to the hard-seeded state; however, this has now been stabilized. The seed has a more rounded appearance than the wild type and is fully permeable to water.

The mutant AM4.16 showed vigorous early growth. The mutant was found to have lost the requirement for vernalization and, as a consequence, lost the rosette phase of growth, which is characteristic of wild *L. atlanticus*. The mutant was earlier flowering, by up to two weeks, but did not produce as many flowers on the main stem as the parent material. This character is controlled by a single recessive gene. White seed and white flowers are common characters produced by mutation. All the above characters have been integrated into a single background giving a fully domesticated type.

9.5.1.6 Lupinus pilosus

Different accessions of wild *L. pilosus* were evaluated for yield in 1992 and 1993 at Mt. Barker, a long-season site in Western Australia. Yields varied from more than 4 tonnes/ha to as little as 2 tonnes/ha, and accessions in morphological Group 130 (Clements et al., 1996) were the highest yielding. This was predictable, as Group 130 had the highest main stem yield, a large number of pods, large seed, and large leaves. Group 131 was the next highest-yielding group. Both 131 and 130 had early rapid growth with early- or mid-flowering times; both characteristics needed to exploit the most from the environment at Mt Barker. Group 132 was not included in the experiment due to lack of nodulation. Accessions from Groups 130, 131, and 129 will be most useful for a breeding program.

Several mutant populations of *L. pilosus* were produced and characters of interest selected (Buirchell, 1999). These included low alkaloids (2 plants), water permeable seeds, white flowers and seeds (1 plant), and determinate plant structure (1 plant). The mutant line PM1.27 has shatter-resistant pods similar to the AM5.1 in *L. atlanicus*, in that the dorsal seam of the pod has fused. Mutant PM1.34 has water-permeable seed. The seed are much smaller than the original parent material. In addition, the pods on PM1.34 are hard and brittle, and the podwalls shrink around the seeds. This pod characteristic, in association with the nonshattering gene in PM1.27, gives the resulting plants excellent nonshattering characteristics. PM7-so is also a water-permeable mutant, but it has retained all the characteristics of the parent line. This line has been crossed with the white-seeded PM9.1 and the water-permeable, white-seeded lines selected. PM1.26, PM1.55, and PM1.38 are low-alkaloid mutants. PM9.1 has a white seed coat and could be used as a genetic marker to differentiate wild types from domesticated types. PM1.60 has a smooth seed coat, having lost the rough characteristic, and can be useful in reducing the wear and tear on machinery that would occur if a rough seed coat was retained. The domestication genes have now been combined, and fully domesticated lines have been produced and yield trials conducted at a number of different environments.

9.5.1.7 Lupinus cosentinii

Several mutant characters were induced by Gladstones including sweetness genes (Gladstones and Francis, 1965b) and early flowering genes (Gladstones, 1958). Two natural mutations were found, which had pod-shattering resistance. Gladstones (1967) showed that the *macer* gene was missing the inner lining of the pod, while *coniunctus* gene was sealed at the dorsal seam of the pod. Both genes created some resistance to pod shattering, but combined together, they produced a very strong resistance to shattering.

The soft-seeded character, which is controlled by a single recessive gene, although imparting soft-seededness to the plant, also causes a penalty in yield and seed size. It is not a very satisfactory gene because of these deleterious effects. The low alkaloid cv. Erregulla was experimentally releaseed in Western Australia, but the genotype was found to be highly susceptible to aphids.

9.5.1.8 Lupinus mutabilis *and Other New World Species*

Lupinus mutabilis can be treated as an outcrossing species based on several recorded estimates of outcrossing such as: from 4 to 11% (Blanco, 1982), 6.4 to 13.4% (Röemer and Jahn-Deesbach, 1988), 16.6 to 58.8% (Caligari et al., 2000), 10% and 19% on main stem and lateral branches, respectively (Gnatowska et al., 2000). Blanco (1982) hypothesized that landrace and wild genotypes of *L. mutabilis* are likely to outcross at higher percentages than the figures he quoted, which were for more domesticated types. Römer and Jahn-Deesbach (1988) noted heterosis expressed as increased early vigor and increased average height of plants in F_1 progeny from crosses combining low-alkaloid and early-flowering traits. Exploitation of heterosis could be worth investigating for *L. mutabilis*, either in terms of maintaining a degree of heterozygosity in cultivars or through use of male sterility. Male sterility identified in a few plants of a breeding line is currently under investigation for its potential to facilitate crossing, which is inherently difficult in *L. mutabilis* compared with the other crop lupin species (Clements, unpublished, 2001). Siamasonta and Caligari (1999) also found that in *L. mutabilis*, growth of embryos was significantly slower after hand pollination by other *L. mutabilis* genotypes, compared with *L. albus* genotypes. Blanco (1982) noted that the occurrence of male sterile plants was not uncommon in Peruvian crops of the species and Pakendorf (1970) examined the mechanisms of the phenomenon in breeding material.

Lupinus mutabilis has a day neutral photoperiod response (Hackbarth, 1936) and is responsive to vernalization (Hardy et al., 1998). A wide range of flowering times can be found in the species, which will be useful for breeding (Table 9.5). There are reports of genotypes that were selected to be harvestable within a three-month growth period in some environments (Binsack, 1991). The indeterminate nature of *L. mutabilis* results in production of many branch orders under conditions where moisture is not limiting (Röemer and Jahn-Deesbach, 1988). Plants can produce up to 52 branches and plant height can range from 0.23 to 2.25 m (Galdos, 1982). Branching structure in *L. mutabilis* is affected by environment but is highly heritable, and this facilitates evaluation of germplasm at few sites (Hardy et al., 1998). Genotypes with restricted branching have been artificially induced. Examples of these are reported by Sawicka (1991) and Stawiski and Rybiski (2001), who produced a semideterminate mutant and a semidwarf mutant, and by Römer and Jahn-Deesbach (1986). The determinate trait of the mutant ‘KW 1’ from the German work was found to have a monogenic recessive inheritance (Caligary et al., 2000). Some limiting agronomic factors identified in *L. mutabilis* have been shown to include low leaf area development early in the growth cycle, a lower conversion of PAR into dry matter, and a short duration where LAI was greater than 2.5, resulting in lower yield potential (Hardy et al., 1997, 1999). Main stem height and days to flowering were related to the number of main stem leaves, but additional environmental factors also

influenced days to flowering (Hardy et al., 1998). Heritability of days to flowering was much lower than was observed in other species, such as *L. albus*.

Seed protein and oil contents in germplasm collections of *L. mutabilis* can vary widely. Values ranging from 35 to 50% for protein (mean of 42%) and from 13 to 24% (mean of 20%) for oil have been demonstrated (Römer and Jahn-Deesbach, 1986; Caligari et al., 2000). Wild genotypes were noted as having the highest protein contents (Mujica et al., 2004). The oil has a high content of polyunsaturated fatty acids (Gross, 1986). The correlation coefficient between protein % and oil % has been reported to be −0.71, calculated over 217 ecotypes (Perez et al., 1984), although a much lower correlation was found in a group of breeding lines, indicating that it was possible to combine high levels of both (Röemer and Jahn-Deesbach, 1988). The correlation between seed yield and oil and seed yield and protein was at −0.50 and 0.41, respectively (Perez et al., 1984). Other desirable seed-quality attributes, such as seed coat proportion of whole seed, can reach as low as 10.6% (mean of 12.9%) (Clements et al., 2004a), and levels of NSPs in whole seed are only around 9% (Brillouet and Riochet, 1983).

Low-alkaloid plants have been identified through mutation breeding (Pakendorf, 1974; Williams et al., 1984) and by selection of spontaneous mutants as in the case of cv. Inti, which was developed from Peruvian germplasm after successive steps of breeding (von Baer and von Baer, 1988). Inheritance of the low-alkaloid trait in cv. Inti is recessive but polygenic in nature, such that only approximately 12% F_2 plants give low-alkaloid seeds (von Baer and von Baer, 1988). Inheritance of pure white seeds is recessive and independent of alkaloid content (Röemer and Jahn-Deesbach, 1988). Rapid Dragendorf test in *L. mutabilis* is often not reliable, since it has been noted that plants at the preflowering stage will often test as "sweet," but when retested during pod set are found to test "bitter" (Römer and Jahn-Deesbach, 1986).

Talhinhas et al. (1999) found that *L. mutabilis* was susceptible to cold and frost and competed poorly with weeds. Variability for cold tolerance, a breeding objective for many countries, has been identified in a small number of breeding lines (Santos et al., 1999). As with the other crop species, anthracnose is one of the main disease concerns. The disease is more severe in warm, humid climates and is widespread in the Andes region. Genotypic variability has been reported (Blanco, 1982), and germplasm from northern Peru ("chocho" type) has been suggested as a source of resistance (Sweetingham, 2000). Resistance to fusarium has been identified (Frey and Yabar, 1983), and one line showing superior resistance to fusarium and viruses was accession K2135 in the VIR collection (Kiselev et al., 1984). *Lupinus mutabilis* is very susceptible to Pleiochaeta root rot and brown spot, but some genetic variation is apparent (Thomas and Sweetingham, unpublished). Limited information suggests that the species is relatively resistant to phomopsis (Jaarsveld and Knox-Davies, 1974). CMV is a moderate problem in *L. mutabilis*, although seed transmission has not been recorded (Jones and McLean, 1989; Jones and Latham, 1996). High resistance to CMV has recently been identified in one landrace accession, P26956, held in the ALC, Perth (Jones and Burchell, 2004). BYMV is a serious problem in *L. mutabilis*, and screening for resistance is *a priority* (Jones and McLean, 1989).

Lupinus mutabilis is a species of great potential, although considerable effort will be required to combine traits to produce fixed low-alkaloid types with suitable phenology, adequate disease resistance, and stable yield and maturity. High yields are achievable in

L. mutabilis but have proven to be unstable in different environments (Caligari et al., 2000), and maturity under humid conditions has been difficult. In Europe, a partially determinate crop with early maturity is likely to be the ideotype for productive *L. mutabilis* crops. In Mediterranean environments, early-flowering genotypes (indeterminate or with mild determinacy) with good early biomass development and a reasonable number of main stem leaves may be the most successful plant type.

For the other New World species of agronomic significance, low-alkaloid forms of *L. polyphyllus* have been bred (Plarre, 1991; Röemer, 1995; Kurlovich, 2002a), including var. Pastwiel, var. Plarre, and var. SF/TA (Gudjónsson and Helgadóttir, 2000), and further development has led to improvements in soft-seededness and reduced shattering for spring and autumn sowing (Chekalin et al., 2001). These domesticated genotypes can be used both for amelioration of eroded land and for grazing. In the case of spring-sown *L. polyphyllus*, there is the need for breeding genotypes that do not require vernalization. Low-alkaloid genotypes of *L. nootkatensis* have also been selected (Plarre, 1991; Gudjónsson and Helgadóttir, 2000) for use as green and processed fodder in Iceland.

9.5.1.9 Developments in Double Haploids in Lupins

Several workers have attempted to develop a double haploid protocol in a range of species, including *L. hartwegii, L. luteus, L. albus, L. angustifolius,* and *L. polyphyllus* (Sator et al., 1983; Sator, 1990; Campos-Andrada and Mota, 1994; Omerod and Caligari, 1994). Culture of isolated microspores of *L. polyphyllus* by Palada and Sator (1981) was unsuccessful. A few plants were generated from the method developed by Sator (1985) using anthers of *L. polyphyllus*. Campos-Andrada and Mota (1994) took *L. hartwegii* anthers with pollen grains at the late tetrad or uninucleate stages of development and cultured them to develop 20-week old calli, which showed some differentiation of small root and shoot primordia-like structures. Their method, however, could not ascertain whether growth originated from pollen grains or from somatic tissue. Working with *L. albus,* Omerod and Caligari (1994) produced embryolike structures from microspores, but the degree of cell division was not confirmed, and later stages were not achieved. Recently, Bayliss et al. (2004), using a "blender method" of isolation of *L. albus* microspores, induced cell division to at least the 16-cell stage of development. Further growth as multicellular microspores has been limited, however, by a strong exine layer that fails to rupture or degenerate. Methods to overcome this in other crop species have not, so far, worked for lupins (Bayliss et al., 2004). The other route used to generate double-haploid plants when anther or microspore culture-based methods have not worked is that of wide-hybridization, such as the wheat×maize system to produce double haploids for wheat-breeding programs (Laurie and Bennett, 1988; Almouslem et al., 1998). This method has not been explored in lupins, but it could arise as a by-product of interspecific crossing research in the genus in the future.

9.5.2 Genomic Relationships, Gene Pools, and Wide Hybridization

Harlan (1984) suggested the phenotypic diversity seen in most cultivated plants is due more to gene regulation than to the appearance of new genes, and this provides a strong case for the exploitation of wild relatives and wider gene pools (through interspecific

crossing) to broaden the genetic base of the crop. Since *Lupinus* is a relatively young crop with respect to its breeding and development, there is still a very large component of the primary gene pool in germplasm collections of the various *Lupinus* crop species yet to be exploited. Gladstones (1974, 1984) noted that the Old World species are separated by profound genetic and, in some cases, reproductive and geographical barriers, particularly among the smooth-seeded species, and between them and the rough-seeded species. Of the 12 described species of the Old World, 3 smooth-seeded species share the same chromosome number—*L. luteus, L. hispanicus,* and L. *micranthus* (2n=52). Within the 7, more closely related Old World rough-seeded species, *L. atlanticus* and *L. princei* (2n=38) and *L. pilosus* and *L. palaestinus* (2n=42) are those with the same chromosome numbers.

9.5.2.1 Genomic Relationships and Gene Pools of Lupins

There is a wide phylogenetic distance between lupin and the cool season food legumes, which include pea, lentil, faba bean, and chickpea. The lupin genome is distinct from these other species in that its chromosome number varies widely (from n=12 to n=26), it expresses a significant number of duplicate loci for relatively conserved isozymes (Wolko and Weeden, 1990a), and it has a smaller DNA content per haploid complement. Compared with other crop species, cytological work in *Lupinus* is relatively deficient, and this is partly attributed to factors such as the large numbers of chromosomes (range from 2n=32 to 52) and similarity in chromosome size and shape, making pairs difficult to identify and to distinguish individual species through chromosome length or arm ratios. The cytological studies that have been done have tended to suggest that lupins may be evolutionary polyploids, but this is still not certain, and the basic number (x) that has been proposed has varied. Dunn and Gillett (1966) defined New World species as having x=6, and the studies of Gupta et al. (1996) indicate the same for the rough-seeded Old World species. Pazy et al. (1977) hypothesized a series of x number from 5 to 13 for the Old World lupin species. The data of Naganowska et al. (2003a), based on one 5S rDNA locus, contradicted the hypothesis of polyploidy but suggested the use of additional repetitive DNA sequences and large DNA fragments to examine the genus further.

Based on the classical definition of Harlan and de Wet (1971)—and on the available taxonomic, crossability, molecular diversity, and other studies for lupins—the following gene pools (GP) for the domesticated *Lupinus* species are suggested:

> *L. albus.* GP1: *L. albus* var. *albus, L. albus* var. *graecus* (wild form); GP2: *L. micranthus;* GP3: all other Old and New World *Lupinus.*
>
> *L. luteus.* GP1: wild forms, landraces, cultivars, 'ssp. *orientalis*'; GP2: *L. hispanicus* ssp. *bicolor* and ssp. *hispanicus, L. angustifolius;* GP3: all other Old and New World *Lupinus*.
>
> *L. angustifolius.* GP1: all wild, landrace and domesticated forms of *L. angustifolius;* GP2: *L. luteus* and *L. hispanicus;* GP3: all other Old and New World *Lupinus*.
>
> *L. cosentinii.* GP1: *L. digitatus, L. atlanticus, L. pilosus;* GP2: *L. palaestinus, L. princei,* and *L. somaliensis;* GP3: all other Old and New World *Lupinus*.

L. mutabilis. GP1: western North and South American New World *Lupinus* with 2n=48; GP2: *L. albus, L. micranthus;* GP3: all other Old World and eastern New World *Lupinus*.

These groups are still speculative in lieu of further crossability studies, especially among the large range of New World species.

9.5.2.2 Wide Hybridization

The first reported attempts at interspecific crossing in the genus are those of Gollmick (1937), whose histological studies and crossing attempts, particularly with *L. angustifolius* and other Old World species, demonstrated that introgression had some potential for success. Further attempts by Jaranowski (1962), using reciprocal crosses among the Old World species, highlighted histologically the degeneration of embryos after early development due to endosperm failure, although the fact that cessation was postfertilization lent some support to continuing this line of research, including developing methods of embryo culture. As early as the 1950s, work on culturing lupin embryos was being carried out and Tomaszewski (1958) reported on methods for embryo culture of several legume species including *L. luteus, L. albus,* and *L. angustifolius*. More recently, work by Williams et al. (1980), Vuillaume and Hoff (1986a, 1986b), Schäfer-Menuhr (1986, 1987, 1989), Podyma et al. (1988), Kasten and Kunert (1991), Kasten et al. (1991), Przyborowski and Packa (1997) have provided further information and, in several cases, progress in methods of embryo culture.

Interspecific crossing research in the *Lupinus* genus following very early work is listed in Table 9.11. Other successful hybridizations among the New World lupin species have been reported, e.g., among *L. ehrenbergii (L. hartwegii), L. bilineatus, L. campestris,* and *L. mexicanus* (Planchuelo, 1994) and between *L. nootkatensis* and *L. polyphyllus* (Gudjónsson and Helgadóttir, 2000). As previously mentioned, Russell, who developed the Russell lupin (Gorer, 1970), carried out crosses between North, Central, and South American species, particularly *L. polyphyllus, L. arboreus, L. laxiflorus, L. lepidus, L. hartwegii, L. nootkatensis,* and *L. mutabilis.*

Kasten et al. (1991), after first developing suitable methods using selfed embryos (Kasten and Kunert, 1991), rescued embryos of *L. mutabilis*×*L. hartwegii* crosses at 20 days after pollination (DAP), and using culture media, brought F_1 plants through to set viable F_2 seeds. The culture media consisted of a liquid over a solid layer of B5 media, with added hormones and amino acids. The hybrid status of the plants was confirmed both by isozyme analysis and by the presence of intermediate morphological characters or transfer of characters more prevalent in the male parent to the hybrids. In the reciprocal cross, *L. hartwegii*×*L. mutabilis,* viable plants were achieved without embryo rescue. In the case of the combination *L. angustifolius*×*L. luteus,* confirmed hybrid F_1 plants were transferred to soil 12 weeks after embryo rescue, but these "experimental plants" died in attempts to adapt them to glasshouse conditions. *Lupinus angustifolius* was found to be the more suitable female rather than in the reciprocal direction. They noted that to increase the chances of obtaining hybrid plants through embryo rescue, it is necessary to rescue embryos at the most advanced stage as possible, but before degeneration of embryo or endosperm has begun. Research in this area with various

genera has often emphasized the importance of experimenting not only with crosses in reciprocal, but also using a range of different genotypes in these combinations as well (e.g., Roy and Gladstones, 1988). Some work has indicated that higher success is obtained

Table 9.11 Attempts at Interspecific Hybridization in Lupins from the Literature

Cross	Stage Achieved, Notes	Reference
L. pilosus×*L. palaestinus*	Sterile F_1 plants	Kazimierski, 1961
L. pilosus×*L. cosentinii*	Shriveled seed (and reciprocal)	Gupta et al., 1994, 1996
L. pilosus×*L. digitatus*	Abscission of pods (and reciprocal)	Gupta et al., 1994, 1996
L. pilosus× *L. atlanticus*	Viable seed (and reciprocal)	Gupta et al., 1994, 1996
L. pilosus× *L. princei*	Shrivelled seed (and reciprocal)	Gupta et al., 1994, 1996
L. pilosus× *L. palaestinus*	Abscission of pods	Gupta et al., 1994, 1996
L. palaestinus×*L. pilosus*	Fertile plants	Pazy, 1981, 1982
L. palaestinus×*L. cosentinii*	No pod formation (reciprocal: pod abscission)	Gupta et al., 1994, 1996
L. palaestinus×*L. digitatus*	Abscission of pods (and reciprocal)	Gupta et al., 1994, 1996
L. palaestinus×*L. atlanticus*	No pod formation (reciprocal: shriveled seed)	Gupta et al., 1994, 1996
L. palaestinus×*L. princei*	Shrivelled seed (and reciprocal)	Gupta et al., 1994, 1996
L. princei×*L. cosentinii*	Shriveled seed (and reciprocal)	Gupta et al., 1994, 1996
L. princei×*L. digitatus*	Shriveled seed (and reciprocal)	Gupta et al., 1994, 1996
L. princei×*L. atlanticus*	Shriveled seed (and reciprocal)	Gupta et al., 1994, 1996
L. atlanticus×*L. cosentinii*	Viable seed (and reciprocal)	Roy and Gladstones, 1988; Gupta et al., 1994, 1996
L. atlanticus×*L digitatus*	Viable seed (reciprocal: abscission of pods)	Roy and Gladstones, 1988; Gupta et al., 1994, 1996
L. digitatus×*L. cosentinii*	Viable seed (and reciprocal)	Roy and Gladstones, 1988; Gupta et al., 1994, 1996

L. cosentinii×*L. digitatus*	Viable seed	Gupta et al., 1994, 1996
L. angustifolius×*L. luteus***	12-week-old “experimental hybrids”	Kasten et al., 1991
L. hispanicus ssp. *bicolor*×*L. luteus*	Fertile plants	Kazimierski and Kazimierska, 1965; Swiecicki, 1988b
L. luteus× *L. hispanicus* ssp. *hispanicus*	Fertile plants, better to use *L. luteus* as female, use of backcrossing to L. luteus	Swiecicki, 1988b; Swiecicki et al., 1999
L. luteus× *L. micranthus*	Embryo died between 8–15 DAP*	Kazimierski, 1988
L. luteus× *L. hartwegii*	Embryos died at early globular stage	Busmann-Loock et al., 1992
L. albus×*L. mutabilis***	Seed without embryos, embryos died (embryo rescue)	Vuillaume and Hoff, 1986a
L. albus var. *albus*×*L. mutabilis*	Viable plants	Sawicka-Sienkiewicz and Brejdak, 1999
L. elegans×*L. mutabilis*	Viable seeds	Huyghe, cited by Römer and Jahn-Deesbach, 1988
L. elegans×*L. mutabilis*	F_1 seeds	Sawicka-Sienkiewicz and Brejdak, 1999
L. pubescens×*L. mutabilis*	F_1 seeds	Sawicka-Sienkiewicz and Brejdak, 1999
L. nanus×*L. mutabilis*	F_1 seeds	Sawicka-Sienkiewicz and Brejdak, 1999
L. polyphyllus×*L. mutabilis*	No pods set	Sawicka-Sienkiewicz and Brejdak, 1999
L. polyphyllus×*L. mutabilis***	Embryo rescue, embryos died	Römer and Jahn-Deesbach, 1988
L. polyphyllus×*L. mutabilis*	Viable plants, use of backcrossing to *L. mutabilis*	von Baer and Barra, 1990; von Baer, 2004
L. hartwegii×*L. mutabilis*	Viable seeds	Kazimierski, 1964b; Schäfer-Menuhr and Busmann, 1987; Kasten et al., 1991Busmann-Loock et al., 1992
L. mutabilis×*L. hartwegii***	Embryo rescue, F_2 seed	Schäfer-Menuhr and Busmann, 1987; Schäfer-Menuhr, 1988; Schäfer-Menuhr et al., 1988; Kasten et al., 1991
L. mutabilis×*L. hartwegii***	Protoplast fusion and embryo culture	Schäfer-Menuhr, 1989
L. mutabilis×*L. hartwegii*	F_1 seeds	Sawicka-Sienkiewicz and Brejdak, 1999

L. mutabilis×*L. polyphyllus*	No pods set	Sawicka-Sienkiewicz and Brejdak, 1999
L. mutabilis×*L. albus* var. *albus*	Viable plants	Sawicka-Sienkiewicz and Brejdak, 1999
L. mutabilis×*L. albus* var. *graecus*	Pods without viable seed	Sawicka-Sienkiewicz and Brejdak, 1999

* DAP=days after pollination; ** Use of methods more advanced than hand-pollination.

when the species with the higher chromosome number is used as the female (Gupta et al., 1996; Sawickia-Sienkiewicz and Brejdak, 1999). Inclusion of more primitive genotypes of the species may also be worthwhile. Applying hormones such as gibberellins, auxins, and cytokinins, and sometimes other nutrients, has been recommended at or soon after the time of pollination (e.g., Singh et al., 1990; Kynast et al., 2001). Other techniques that can be used include chromosome doubling of the obtained hybrids to produce amphidiploids with restored fertility (Jones, 1983) and breeding methods such as single, congruity, or recurrent backcrossing (Swiecicki, 1984; Roy and Gladstones, 1985, 1988; Singh et al., 1990, 1993; Anderson et al., 1996) to enhance fertility or to concentrate the desirable genetic background. At a practical level, breeders can also use repetitive pollination of the female over a series of days to enhance the chances of receptivity of the stigma. Additionally, short to medium term, low-temperature storage of pollen can also facilitate crossing where flowering times of two species are highly disparate (Campos-Andrada, 1999). Schäfer-Menuhr (1988, 1989, 1991a, 1991b) and Wetten et al. (1994) reported on the use of tissue culture to multiply plants from a single tissue source and also regenerated hybrid plants using leaf protoplast culture. These methods, which were developed despite the recalcitrant nature of lupins for tissue culture, could be used to multiply progeny when the number of hybrid plants is very low and at risk of being lost.

The high protein and oil content of *L. mutabilis* has made it attractive as a candidate for introgression with other crop lupin species. Przyborowski and Packa (1997) conducted interspecific crossing and embryo development studies among two Old World *(L. albus, L. angustifolius)* and the New World species *L. mutabilis*. They found that the largest number of developing pods was obtained when *L. angustifolius* was used as the female especially in the cross combination *L. angustifolius*×*L albus*. They succeeded in obtaining plants grown from embryos rescued on MS media (Murashige and Skoog, 1962) for the crosses *L. albus*×*L. angustifolius* and *L. mutabilis*× *L. angustifolius* and each of their reciprocals. The optimum time for rescue was between 10 and 15 DAP or between 15 and 20 DAP in the case of *L. albus*×*L. angustifolius*. The hybrid status of some surviving plants was still to be confirmed.

Sawicka-Sienkiewicz and Brejdak (1999) succeeded in obtaining fertile plants in crosses without embryo rescue in the combination *L. albus* (both var. *albus* and var. *graecus*)×*L. mutabilis*. Several hybrid populations have been brought through to more advanced generations and morphological, cytological, and molecular marker studies have provided some evidence of their hybrid status (Sawicka-Sienkiewicz et al., 1998; Sawicka-Sienkiewicz, 2004). The chromosome numbers of hybrids, which were usually morphologically similar to the female, were found to be the same as *L. mutabilis* (2n=48), irrespective of the female parent. What was notable in these studies was the reasonably

high recovery of viable seeds, being in the order of 7%, based on the number of flowers pollinated. It was noted in this work that *L. albus* tended to be the more suitable female in crosses with *L. mutabilis,* as has been found by others (Siamasonta and Caligari, 1999), where embryo growth rates were faster compared with the reciprocal cross. Further crossing work and characterization of existing lines, in addition to development of molecular methods for confirmation of hybrids, is currently being carried out (Sawicka-Sienkiewicz, personal communication, 2004). Further to this work with *L. mutabilis,* crosses of *L. polyphyllus*×*L. mutabilis* were attempted with the aim of introgressing the nonshattering, high protein and oil contents of the Andean lupin and the comparatively better cold tolerance of *L. polyphyllus* (von Baer, 2004). Both Schäfer-Menuhr and Busmann (1987) and Römer and Jahn-Deesbach (1988) found that success was more likely if *L. polyphyllus* was used as the female. Such bridging between these two species may also facilitate the development of *L. polyphyllus* for grazing.

mosome numbers, the groups are considered to be very homogeneous (as discussed above). The most mutually compatible species in these crossability studies were the group *L. cosentinii* (2n= 32), *L. digitatus* (2n=36), and *L. atlanticus* (2n=38). Although not obtained by Gupta et al. (1996), possibly because of the choice of the single *L. palaestinus* genotype, crosses between *L. pilosus* and *L. palaestinus* (both 2n=42) were obtained by previous workers (Kazimierski, 1961; Pazy et al., 1981, Pazy, 1982) and indicated compatibility between the two. The grouping of *L. cosentinii, L. digitatus,* and *L. atlanticus,* and then of *L. pilosus* and *L. palaestinus,* is supported to varying degrees by previous cytological (Carstairs et al., 1992), nuclear DNA contents (Ghrabi et al., 1999; Naganowska et al., 2003a), ITS data (Ainouche and Bayer, 1999), and seed proteins (Salmanowicz, 1994). The more genetically isolated and morphologically distinct *L. princei* (2n= 38) was found to have larger chromosomes (Carstairs et al., 1992) but an unusually low 2C nuclear DNA content (Naganowska et al., 2003b). Introgression among the rough-seeded lupins should allow the transfer of domestication traits (reduced shattering, permeable seeds, early flowering) and adaptation to fine-textured, neutral to alkaline soils and tolerance to free lime. In several two-and three-way crosses among *L. cosentinii, L. digitatus,* and *L. atlanticus,* a range of univalent frequencies were observed, the highest being between *L. cosentinii* and *L. digitatus,* and usually an intermediate chromosome number resulted (Table 9.12).

The breeding efforts reported in Roy and Gladstones (1985, 1988), Buirchell and Cowling (1992), and Gupta et al. (1996) have allowed the transfer of important domestication genes from *L. cosentinii* and *L. digitatus* into *L. atlanticus*. Table 9.12 shows that the seed permeability gene, early-flowering gene, and one of the genes *(ma)* coding for nonshattering pods were transferred from *L. cosentii* to *L. atlanticus-type* plants. These hybrids had chromosome numbers varying from 34 to 38. Of the two nonshattering genes, only *ma* has been found to be expressed in hybrids with *L. atlanticus* and only where *L. digitatus* was in the pedigree. The reason for this is unknown. A hybrid between *L. atlanticus* and *L. digitatus* has produced an early-flowering hybrid (85E48) that is thermoneutral. Neither of the parents exhibit any of the early-flowering

9.5.2.2.1 Interspecific Crossing in the Development of the Rough-Seeded Lupins

Interspecific crossing among the rough-seeded lupins has been reasonably successful with fertile hybrids now at advanced stages of breeding (Gupta et al., 1996). Even with their range of chro-

Table 9.12 Chromosome Numbers, Genes of Interspecific Hybrids and Genes Transferred from *Lupinus cosentinii* cv. Erregulla-Soft into *Lupinus atlanticus*

Cross	2n	Genes	Pedigree
85E10	34	*so*	*atl/cos*
85E48	?	*xe*	*atl/dig*
88E63	36	*sw2 so*	(*atl*/Erg-so//*atl-sw1*)//*atl/atl-sw2*
89E29	36	*sw xe*	*atl/dig//cos*
89E30	36	*sw so*	*atl/dig//cos*
89E50–15	36	*sw1 ma*	*atl/atl//*(*atl/dig//*(*atl//a*tl/Erg-so))
89E51–11	38	*sw1 xe*	*atl/dig*
89E29–7	38	*sw2 xe*	{(Erg-so/*atl*/)/4/Erg-so//*atl*/Erg-so/3/Erg-so}/*{(atl/dig)/(atl/atl-sw2)}*
92E02	36/38	*sw2 ShR1 so*	*atl/cos//atl*
93E02	36/38	*so shr1 sw2*	88E63/AM5. 1-*Shr1*
Erg-so	32	*so ma/co sw xe*	*L. cosentinii* cv Erregulla-soft

Note: so=water-pe ermeable seeds, *xe*=early flowering, *sw1*=low alkaloid, *sw2*=low alkaloid, *ma*= reduced shattering pods, *atl=L. atlanticus, dig=L. digitatus, cos=L. cosentinii*.

characteristics expressed by this hybrid. The earliness is controlled by a single dominant gene. This gene has also been transferred to other interspecific hybrids. *L. atlanticus* is now considered to be fully domesticated, as the hybrid 93E02 has the three main domestication characteristics of low alkaloids, nonshattering pods, and seed permeability, with the latter coming from *L. cosentinii* cv. Erregulla-soft.

Many reports of successful wide hybridization in other genera have highlighted a range of more advanced methods for validating the true hybrid status of progeny and to track the transfer of one genome to another. These include spectral karyotyping, banding, fluorescence staining (e.g., DAPI), genomic *in situ* hybridization (GISH), and fluorescent *in situ* hybridization (FISH) (Jiang and Gill, 1994; Yan et al., 1999). The use of FISH has already begun in *Lupinus* with rRNA genes, where it was possible to distinguish several pairs of chromosomes in *L. luteus*, *L. hispanicus*, and a hybrid between them

(Naganowska et al., 2003a). The studies also showed conservation of the 5.8S genes (from *Vicia faba*) among each of *L. cosentinii, L. pilosus, L. luteus, L. mutabilis, and L. hispanicus* (Naganowska and Zielinska, 2002). Even more recent is the method of primer *in situ* labeling (PRINS), which is an alternative to FISH but has higher sensitivity and other practical advantages (Kubaláková et al., 1997; Naganowska, 2001). With evidence suggesting that *Lupinus* species could be evolutionary polyploids (Pazy et al., 1977; Plitmann and Pazy, 1984; Wolko and Weeden, 1989; Gupta et al., 1996; Wolko, 2001), hybridization events are likely to yield a range of variable progeny, and application of GISH and FISH methods will be extremely useful to track the transfer of whole genomes or proportions of them.

9.5.2.2.2 Somatic Embryogenesis

Interspecific hybridization can also be accomplished through somatic hybridization. Protoplast fusion research in lupins has been reported by Gleba et al. (1986) and Schäfer-Menuhr (1986, 1991a, 1991b), and Wetten et al. (1999). Fusion of cells derived from leaves of the *L. mutabilis* ×*L. hartwegii* hybrid and cell suspension cultures of *L. polyphyllus* were subject to electrical fusion, and plants were regenerated successfully. Electrical fusion was more successful than with PEG solutions, and use of protoplasts that differ morphologically enabled clearer identification of hybrid protoplasts.

In view of the progress made so far in interspecific crossing in lupins, there appears to be good scope for further introgression of traits to crop species with already established agronomic characteristics. Potential for as-yet-unattempted species cross combinations may be worthwhile. For example, with the reported genetic similarity of *L. gibertianus-L. linearis* complex to *L. angustifolius* (Planchuelo-Ravelo, 1991), *L. bandelierae and L. albescens* to *L. albus* and *L. paranensis* to *L. luteus*, and *L. mutabilis* to the western New World species (especially to those in North America) (Käss and Wink, 1994; Dunn, 1984; Swiecicki et al., 2000), lends support to potential introgression among those species. Prospects for interspecific crossing with *L. mutabilis* are promising. Candidates for interpspecific crossing among possible relatives of the Andean lupin as inferred by Tapia and Vargas (1982) and others are: *L. bogotensis, L. cruckshanskii, L. praestabilis, L. aridulus, L. biinclinatus, L. calcencis, L. condensiflorus, L. cuzcencis, L. malacotrichus, L. microphyllus, L. paniculatus, L. praealtus, L. semiprostratus, L. chlorolepis*, and *L. tomentosus*. Hybridization between *L. micranthus* and *L. albus* and *L. luteus* may also be possible, based on chromosome number or phylogenetic grouping. *L. micranthus* has some tolerance to calcareous soils (Gladstones, 1974) and has higher oil content in seed than *L. luteus* (Gladstones and Crosbie, 1979), and it might be possible to transfer these characters to *L. luteus* through interspecific crossing. Exploring the use of *L. hispanicus* for *L. luteus* crop improvement may also provide new benefits to yellow lupin breeding programs. Further work is required, particularly in embryo culture and successful transfer to soil—and also in the application methods for proving the hybrid status of progeny.

9.5.3 Molecular Markers and Mapping

Marker-assisted selection (MAS) is a tool of increasing importance in plant genetic improvement programs (Mohan et al., 1997). The advantages of MAS are very attractive to plant breeders, and include early generation selection, differentiation of homozygous individuals from heterozygous individuals, and selection based on genotype rather than phenotype. Multiplexing enables the simultaneous tracking of several traits of interest (Tanksley et al., 1989). Enormous investment has been directed toward research on MAS worldwide in the last two decades. However, examples of large-scale marker implementation in plant breeding are few, and MAS leading to release of new cultivars is rare (Young, 1999).

The ideal traits suitable for MAS are those that are of substantial economic importance, are controlled by major genes, and are difficult or expensive to assess by conventional means (Kumar, 1999; Young, 1999; Eagles et al., 2001). To warrant the use of MAS, it must be more cost-efficient than traditional glasshouse or field-based selection and have the capacity to deal with large numbers of samples. In lupin, traits desirable for marker development include anthracnose resistance, for which there is no reliable seedling test; phomopsis stem blight resistance, for which phenotyping relies on microscopic examination (Williamson and Sivasithamparam, 1994), and high sulfurprotein in *L. angustifolius* and CMV resistance in *L. luteus*, for which conventional tests are expensive. Other potential advantages to Australian breeding programs would be the ability to trace rust and fusarium wilt resistance, which is otherwise unachievable as the diseases are not present in Australia, and resistance to aphid colonization and feeding damage are difficult to screen. DNA-hybridization-based RFLP markers are not simple for routine screening because they require large amounts of high-purity DNA and laborious procedures of southern hybridization (Gupta et al., 1999). RAPD markers (Williams et al., 1990) are not reliable and reproducible (Provan et al., 1999). Many PCR-based markers originated from multilocus fingerprinting techniques (Brugmans et al., 2003), such as AP-PCR marker (Welsh and McClelland, 1990), RAPD markers and AFLP markers (Vos et al., 1995), which are nonsequence-specific. The association of such a nonsequence-specific marker to a gene of interest cannot be unambiguously confirmed on various breeders' lines, because there is always a possibility of the presence of a band from a different locus in the genome but with the same molecular weight (Gupta et al., 1999). Therefore, nonsequence-specific markers should be converted into "sequence-specific markers" (Shan et al., 1999), sometimes termed "locus-specific markers" (Brugmans et al., 2003), before they can be implemented in breeding programs.

The ideal markers amenable to large numbers of samples with low running costs are sequence-specific PCR markers, such as sequence-tagged microsatellite site (STMS) markers (Beckmann and Soller, 1990), sequence characterized amplified region (SCAR) markers (Paran and Michelmore, 1993), sequence-tagged site (STS) markers (Olson et al., 1989), allele-specific PCR (AS-PCR) markers (Khan et al., 2000), and single nucleotide polymorphism (SNP) markers (Kanazin et al., 2002). PCR-based sequence-specific markers can be obtained either by cloning and sequencing genomic DNA (such as STMS marker and SNP markers), which is expensive and labor-intensive, or by converting nonspecific markers obtained from generic DNA fingerprinting methods

(Yang et al., 2002). The DNA fingerprinting method MFLP (Yang et al., 2001) is capable of producing a larger number of SSR-related polymorphisms, and many MFLP polymorphisms can easily be converted into simple PCR-based markers desirable for marker implementation. The MFLP method has played a pivotal role in marker development in lupin (Yang et al., 2001, 2002, 2004).

Phenotyping the segregating F_2 progeny of resistant×susceptible crosses was used successfully in developing markers for phomopsis stem blight resistance in lupin (Yang et al., 2002). However, F_8 derived recombinant inbred lines (RILs) were more preferable than F_2 because they are "immortal" populations and are advantageous both in phenotyping and genotyping (Loudet al., 2002). Several lupin RIL populations have been established at the Department of Agriculture, Western Australia, including one RIL population derived from a domesticated×wild cross (83A:476× P27255), which is useful for marker development on domestication genes in *L. angustifolius*. Another RIL population derived from Unicrop×Tanjil is suitable for marker development for several agronomic traits in *L. angustifolius;* the third, RIL population of *L. albus*, is derived from Kiev Mutant×P27174. Finally, a RIL population of L. *luteus* is derived from Wodjil×P28213.

There are two approaches in molecular marker development: the mapping-based approach, and the specific marker development approach. Molecular mapping involves the use of a large number (>100) of segregating progeny to generate as many molecular markers as possible. These markers are lined up into linkage groups based on statistical analysis using programs such as MapMaker (Lander et al., 1987) or MapManager (Manly and Elliot, 1991), and each linkage group represents one pair of chromosomes in a genome. A complete molecular map should have the same number of linkage groups as the haploid chromosome numbers in lupin. For example, *L. angustifolius* (n =20) should show 20 linkage groups. Molecular mapping has the advantage of tackling many genes simultaneously, provided that these genes are polymorphic on the two parental plants from which the segregating population is created. However, the construction of a saturated molecular map requires an enormous amount of work. Furthermore, the usefulness of a molecular map depends on the quality of the markers being used in the mapping. Molecular maps based on sequence-specific markers and locus-specific markers have the advantage of locus identity certainty and are transferable to other crosses, at least within the species (Qi et al., 1996). However, if the markers on the map are nonsequence specific, such as RAPD markers or AFLP markers, the map and the markers cannot be unambiguously applied to other crosses that involve different parental cultivars (Gupta et al., 1999).

Some preliminary work on constructing genetic maps for lupins has been reported. Several groups are now working in this area, and results are expected to emerge over the next few years. Gilbert et al. (1999) and Geoffray et al. (2001) each reported work on the development of molecular markers using RAPD, ISSR-PCR, and AFLP techniques for *L. albus*. In addition to constructing a preliminary map using single-seed descent lines, the work was designed to identify molecular markers associated with QTLs for tolerance to alkaline-induced chlorosis (Rogers et al., 2001, 2004). Preliminary molecular maps have been constructed in *L. angustifolius* using RAPD markers (Wolko and Weeden, 1994; Kruszka and Wolko, 1999), AFLP markers (Scobie et al., 2002), and RFLP using soybean and lupin-derived probes (Nelson et al., 2004). Recombinant inbred lines have

also been created in *L. albus* using genetically differing parents—a Turkish indeterminate, bitter accession, and an autumn-sown dwarf determinate low-alkaloid line (Huyghe, 2004).

The specific marker development approach of Yang et al. (2002) uses a small number (10 to 20) of F_2 plants (or RILs) from a cross to search for candidate markers. These selected plants represent the variation of the trait of interest, for example, half the number of plants are resistant to a disease, while the other half of the plants are susceptible. DNA from each group of plants can be pooled as "bulked segregation analysis" (BAS) (Michelmore et al., 1991), which further decreases the amount of work required. In our work on lupin marker development, we prefer to treat each individual plant separately to avoid the detection of false positive markers (Yang et al., 2002, 2004). Once a candidate marker is identified, the marker is converted into a simple PCR-based marker and is subsequently verified on a larger (>150 individuals) population of F_2 or RILs to confirm the linkage between the marker and the gene of interest. By applying the specific marker development strategy, two implementable markers were established tagging a gene conferring resistance to phomopsis stem blight disease in *L. angustifolius* (Yang et al., 2002). Three implementable markers were developed tagging a gene for high level of anthracnose resistance in *L. angustifolius* (Yang et al., 2002; You et al., 2004). In addition, one candidate marker linked to a gene for anthracnose resistance in *L. albus* and three candidate markers tagging a rust resistance in *L. angustifolius* were identified (Yang, unpublished data).

The phomopsis resistance marker (Yang et al., 2002) and the anthracnose resistance marker (Yang et al., 2004) can be multiplexed in PCR. Marker-assisted selection is now integrated into lupin breeding in Australia, which screened more than 7500 breeding materials in 2003. It is important to appreciate that very few molecular markers are "perfect markers" (Yan et al., 2003), where recombination does not occur between the marker and the trait of interest (Eagles et al., 2001). The majority of molecular markers are "imperfect," where a certain genetic distance exists between the marker and the gene. In this case, the presence of a marker may not necessarily imply the presence of the gene (Sharp et al., 2001). In *L. angustifolius*, one of the markers called "AntjM1" is 3.5 centiMorgan (cM) from the *Anr1* gene, conferring high level of anthracnose resistance in cultivars Tanjil and Wonga (Yang et al., 2004). However, other cultivars such as Gungurru, Yorrel, Merrit, Belara, and Quilinock also exhibit the resistance marker band even though they do not have the *Anr1* gene (Table 9.13). As a consequence, marker "AntjM1" cannot be used for MAS in crosses where these cultivars are used as parents.

The potential complication from genetic recombinations leads to the need for "marker validation" (Sharp et al., 2001) to verify the presence of the target gene and its linkage to the intended marker in breeders' lines. Marker validation defines which crosses in a breeder's program can be screened by a particular molecular marker for MAS (Sharp et al., 2001). In MAS, the closer the marker is to the gene, the more accurate and useful in molecular breeding. The marker "AntjNBS1" is 2.3 cM to the anthracnose resistance gene *Anr1* in *L. angustifolius* (You et al., 2004). Marker AntjNBS1 and AntjM1 are on the opposite sides flanking the *Anr1* gene on the chromosome. With marker AntjNBS1, all commercial cultivars not having the *Anr1* gene showed the susceptible marker

Table 9.13 Disease Phenotype and Marker Scoring Commercial Lupin Cultivars for Anthracnose Resistance Caused by *Colletotrichum gloreosporiodies*

Cultivars	Disease Phenotype	Marker "AntjM1[a]	Marker "Antj NBS1"[a]
Unicrop	S	M^S	M^S
Illyarrie	MR	M^S	M^S
Yandee	S	M^S	M^S
Danja	S	M^S	M^S
Gungurru	S	M^R	M^S
Yorrel	S	M^R	M^S
Merrit	S	M^R	M^S
Myallie	S	M^S	M^S
Kalya	MR	M^S	M^S
Wonga	R	M^R	M^R
Belara	S	M^R	M^S
Tallerack	S	M^S	M^S
Tanjil	R	M^R	M^R
Quilinock	S	M^R	M^S

[a] MR=Showing homozygous resistance marker band; M^S=showing homozygous susceptible band.

bands (Table 9.13). As a result, the number of crosses desirable for MAS increased from 23 to 93 with the introduction of the marker AntjNBS1, compared with the old marker AntjM1, in 2003. Furthermore, co-dominant markers are more useful than dominant markers. The two markers for phomopsis resistance (Yang et al., 2002) and the two markers for anthracnose resistance (Yang et al., 2004, You et al., 2004) in *L. angustifolius* are all co-dominant, which made it possible to select only the plants with homozygous resistance marker bands in lupin breeding. One of the most useful applications of molecular markers is marker-assisted accelerated backcrossing to improve an existing cultivar that is deficient in one trait controlled by a major gene. The cultivar is crossed with a plant as the donor of the desirable trait. Three or four successive backcrossings are conducted with the cultivar to be improved as the female parent. A molecular marker is employed to ensure that only the plants with desirable markers are selected and used as pollen donors in each backcrossing cycle. The marker is also used to test the progeny of the final backcrossing to identify the individual plants with homozygous marker bands for the trait. The cultivar Quilinock is the highest yielding *L. angustifolius* among all commercial cultivars released in Australia before 2004. However, it is highly susceptible to anthracnose disease. The anthracnose resistance marker

"AntjNBS1" is now being utilized to incorporate the anthracnose-resistance gene *Anr1* from Tanjil into Quilinock background at the Department of Agriculture, Western Australia, using the marker-assisted accelerated backcrossing strategy.

9.5.4 Genetic Transformation

Genetic transformation offers the opportunity to introduce traits into lupin that are not available in the lupin germplasm. To date, the modifications made to crops using this technology have been relatively simple and mostly achieved by insertion of a single gene. However, with the advances that are occurring in genome mapping and sequencing, and the use of funtional genomics techniques to understnad the genes and gene networks that have been identified, the possibilities for genetic manipulation of crop plants are constantly expanding. Engineering of eomplete metabolic pathways is becoming a reality (Trethaway, 2004) and some of the advances in this area may well be applicable for lupin.

Like many other legumes, lupins have proven difficult to transform, but in the last 10 years, transformaion systems have been developed and procedures improved such that the transformation is a routine procedure at least for *L. angustifolius*. Work in this area has concentrated on improving seed characteristics and disease resistance, but transformation is also being used as a tool to study processes such as pod set and seed development.

9.5.4.1 Transformation Methods

Although there have been numerous reports of transformation of particular cells or tissues in different lupin species, stable transformation has been achieved in three lupin species, *L. angustifolius* (Pigeaire et al., 1997; Molvig et al., 1997), *L. luteus* (Li et al., 2000), and *L. mutabilis* (Babaoglu et al., 2000). In 1997, two different methods for transformation of *L. angustifolius* using *A. tumefaciens* as a vector and the *bar* gene (which confers resistance to the herbicide Liberty) as a selectable marker were reported (Molvig et al., 1997; Pigeaire et al., 1997). The method developed by Molvig et al. (1997) used slices of the embryonic axis isolated from immature seeds as explants for transformation. Regeneration was achieved via organogenesis. The method is no longer used, as transformation frequency was low (0.01%), and it was cultivar-dependant with transformed plants recovered only from the cultivar Warrah. The method developed by Pigeaire et al. (1997) uses the shoot apex of embryos isolated from mature seeds as the explant for transformation. The apex is wounded with a needle before cocultivation with *A. tumefaciens*. Ag10 is the most effective *A. tumefaciens* strain for infection of lupin explants. Transformants are regenerated from axillary buds. Since this does not involve a *de novo* regeneration step (the axillary buds exist at the time of explant preparation) chimeric shoots may be produced, and if the germ cells are not transformed, the transgene is not transferred to progeny. However, transgenic progeny have been recovered from approximately 30% of transformed T_0 shoots—a satisfactory result. The method is used routinely for transformation of a range of *L. angustifolius* cutivars, with transformation frequencies ranging from <1% for cv Tanjil to 3% for cv Merrit. While the original shoots had to be grafted onto a nontransgenic root stock, growth of shoots on IBA (3mg/L) is now used to promote root growth before shoots are transferred to the

glasshouse (Atkins et al., 1998). This method has been modified and used in transformation of *L. luteus* (average transformation 0.27%) (Li et al., 2000) and a number of other legumes including lentil, chickpea, pea, and faba bean (Hamblin et al., 1998). Attempts have been made to transform *L. albus* but without success (Atkins and Smith, 2003).

The shoot apex was also used as an explant for transformation of *L. mutabilis* (Babaoglu et al., 2000). The initial cell layers of the apex were removed prior to co-cultivation with *A. tumefaciens*. After infection, buds developed at the periphery of the apical meristem and transgenic shoots were derived from these. All the transgenic plants described by Babaoglu et al. (2000) originate from the same explant and are likely to represent a single transformation event, so it is not possible to gauge the effectiveness of the protocol.

Freedom to operate issues associated with use of the *bar* gene as a selectable marker and its presence in lines to be commercialized have forced some changes to the protocol for transformation of narrow leafed lupin. Initially, the *nptII* gene (which confers resistance to kanamycin) was tried as an alternative selectable marker. Although transgenic shoots were obtained (Molvig et al., 2003), the negative public perception of plants carrying antibiotic resistance meant that groups working on transformation of lupins in Australia now use twin T-DNA constructs for transformations where the product may be commercialized. In this method, the selectable marker and gene of interest are carried on two separate T-DNAs (the region transferred by *Agrobacterium* into the plant) that may integrate into the plant genome at different positions. This means that after selection of transgenic shoots (T_0 generation), segregation of the marker gene away from the gene of interest allows markerfree transgenic plants to be recovered in the T_1 generation. Although use of twin T-DNAs increases the amount of work required to produce a transgenic line, use of this technology to produce plants carrying only the gene of interest reduces the freedom to operate, and creates regulatory and public-perception problems that can impede release of a commercial product.

In addition to stable transformation, transformation of roots has been achieved in a number of species, including *L. albus, L. polyphyllus* (Mugnier, 1988; Berlin et al., 1991), *L. hartwegii* (Berlin et al., 1991) and *L. mutabilis*, through infection with *A. rhizogenes* (Babaoglu et al., 2004). Much higher concentrations of isoflavones were produced in the transformed roots of *L. mutabilis* than in nontransformed roots, and these levels could be sustained over a long period of time (Babaoglu et al., 2004). This makes them an ideal source for commercial production of isoflavones.

9.5.4.2 Modified Traits

For *L. luteus* and *L. mutabilis*, there are no publications describing transgenic lines after the original publications. Research involving transgenic *L. angustifolius* has been concentrated in three main areas—herbicide resistance, improved seed quality, and disease resistance (Hamblin et al., 1998). Narrow-leafed lupins resistant to the herbicide Liberty (BASTA or phosphinothricin) were produced by Pigeaire et al. (1997) through introduction of the *bar* gene. These lines showed significant resistance to the herbicide, and in field trials, yields were equivalent to the parent cultivar Merrit (Atkins and Smith, 2003). Although they showed some potential for weed control in the wheat-lupin

rotations used in Western Australia, commercial development was blocked due to intellectual property problems associated with use of the *bar* gene.

As a source of protein in animal feed, lupin seeds are deficient in sulfur-containing amino acids. Molvig et al. (1997) addressed this issue by introducing the sunflower seed albumin (SSA) gene, which encodes a rumen-stable protein rich in methionine and cysteine into *L. angustifolius* cv Warrah. Seed-specific expression of the SSA gene resulted in lupin seeds that had 94% more methionine but 12% less cysteine than nontransgenic seeds, with an overall 19% increase in the total seed sulfur content. The reduction in cysteine in the transgenic seed probably occurs as a result of reduced accumulation of one of the endogenous storage proteins that contains a high proportion of cysteine. These results suggest that, although more sulfur can be incorporated into seed storage protein by increasing the sink for sulfur (by expressing SSA), the ability of the seed to import sulfur ultimately limits the amount of sulfur that can be accumulated (Tabe and Droux, 2002). It should be borne in mind that the levels of sulphur amino acids in transgenic cv. Warrah have increased only to levels already existing in the nontransgenic cv. Kalya.

Feeding trials comparing the nutritive value of SSA expressing transgenic seed to that of the parent cultivar have been done in rats (Molvig et al., 1997), sheep (White et al., 2000), broiler chickens (Ravindran et al., 2002), and fish (Glencross et al., 2003). There were no adverse effects from using transgenic seed as feed for animals. In the rat and sheep trials, the transgenic seed gave a significant increase in live weight gain when compared with the nontransgenic seed (Molvig et al., 1997, White et al., 2001), and for sheep, there was an 8% higher rate of wool growth. Use of transgenic lupins in feeds for broiler chickens was shown to be able to reduce the requirement for supplemental methionine in bird diets (Ravindran et al., 2002). For fish, there appeared to be little value in the use of high-methionine lupins as feed (Glencross et al., 2003). These results highlight the different nutritional requirements of different animals.

Seed meal from high-methionine lupins has also been tested as a vaccine to suppress experimental asthma in mice (Smart et al., 2003). This was tested, as SSA has been suggested as a potential allergen. Mice fed seed meal from the transgenic lupin were less likely to develop the symptoms of experimental asthma when challenged with SSA than those fed meal from nontransgenic lupins. These results support the theory that plant-based vaccines may be therapeutic for protection against allergic diseases.

The original transgenic line carrying the SSA gene also contained the *bar* and GUS genes (encoding B-glucuronidase). To produce plants that could be released commercially, work is continuing to produce marker-free lupins expressing SSA, which have equivalent or higher levels of sulfur amino acids in their seeds (Molvig, Higgins unpublished data).

A number of different genes are currently being tested for their efficacy in improving resistance to fungal and viral diseases in lupins (Wylie, Barker, and Smith, unpublished data). The most promising results are for resistance to the bean yellow mosaic virus (BYMV). A nontranslatable inverted repeat of part of the RNA-dependent RNA polymerase gene segment of BYMV has been expressed in narrow-leafed lupin. Three lines carrying this synthetic resistance gene have been identified, which are immune to infection (Wylie et al., unpublished data). These lines are still in the early stages of development, and further testing will be required to confirm their resistance.

The *ipt* gene has been expressed in narrow-leafed lupin with a flower-specific promoter to increase cytokinin levels during pod set in an attempt to reduce pod abortion. The resulting transgenic plants have patterns of branching quite different from that of the parent cultivar. While in nontransgenic lupins branches develop only from the lowest few axillary buds and from three or four buds directly below the main stem inflorescence, in the IPT transgenic lines branches develop from all axillary buds. The implications of the modification on yield are currently being investigated.

In conclusion, tranformation protocols are available for *L. angustifolius, L. luteus,* and *L. mutabilis;* however, only the *L. angustifolius* method is being used routinely to produce transgenic plants. The most significant practical progress has been in improving seed quality and, more recently, in developing virus-resistant lupins.

9.5.5 Mutation Breeding

The majority of the important domestication traits for lupins were the result of spontaneous natural mutants. With the exception of *L. cosentinii,* all low-alkaloid selections in the crop lupin species, *L. albus, L. luteus,* and *L. angustifolius,* were natural occurrences. Both natural and induced low-alkaloid forms of *L. mutabilis* have been identified. For *L. luteus* and *L. angustifolius,* the frequency of naturally occurring low-alkaloid plants was 1 per 250,000 plants (Pakendorf, 1974). The inheritance of most of the character traits were of simple Mendelian type and conditioned by major genes. Mutagenesis can increase mutation rates by 10 to 100 (Micke, 1993), and in most cases the mutant genes have been recessive for the selected trait (Gottschalk and Wolff, 1983). This has been invariably true for lupin-induced mutant genes, and they have segregated in simple Mendelian diploid fashion, despite the assumption that they are evolutionary polyploids. Keeping this in mind, selection of mutants at higher frequencies could be achieved using M_3 generations rather than the M_2. Examples of mutant traits selected in lupin genotypes are: the *efl* gene for earlier flowering in *L. angustifolius* cv. Chittick (Francis and Gladstones, 1963; Gladstones and Francis, 1965a), low alkaloid *(tantalus)* in *L. angustifolius* (Zachow, various traits in *L. albus* cv. Kiev Mutant and cv. Neutra early flowering in cv. Start (Golovchenko, 1982; Plarre, 1982; Vavilov and Gataulina, 1984; Gataulina, 1994), low alkaloid *(sw),* early flowering *(xe)* and white flowers and seeds *(wfs)* in *L. cosentinii* cv. Erregulla (Gladstones and Francis, 1965b; Gladstones, 1994), restricted branching *(ep1)* in *L. albus* and several other growth habit types in *L albus* and *L. luteus* (Mikolajczyk et al., 1984; Micke and Swiecicki, 1988), round seeds *(plenus)* in *L. albus* (Plarre, 1991), and dwarfism (Harzic and Huyghe, 1996) in *L. albus*. All of the domestication traits in *L. atlanticus* and *L. pilosus* have come from mutation breeding.

The following are examples of methods and frequency of traits in mutation of lupins. In the case of *L. mutabilis* using 40 Kr gamma irradiation, a frequency of one low alkaloid plant per 3887 plants was produced (Pakendorf, 1974). Buirchell (1999) used a method of presoaking 4000 *L. pilosus* seeds in water for 4 hours, followed by 0.5% v/v ethyl methane sulphonate (EMS) for 12 hours and postwashed thoroughly in water. Out of approximately 7000 M_2 plants, 2 low-alkaloid plants and 1 plant each having permeable seed coat and white flowers were identified. The application of mutation breeding in the rough-seeded lupins is covered in the Conventional Breeding section above. Clements and Atkins (2001) presoaked 4500 seeds of *L. angustifolius* in running

tap water at 16°C, followed by 6 hours air-bubbled 0.43% v/v EMS in 0.1 M potassium phosphate buffer (pH 7.0) and postwashed for 10 hours in running tapwater. From an M_2 plant population of approximately 24,000, three nonabscission plants were selected. This method has been further refined as follows for *L. angustifolius:* presoak 10,000 seeds for 3.5 hours in bubbled tapwater, followed by 14 h with 0.3% v/v EMS in 0.025 M potassium phosphate buffer (pH 7.0) with air bubbling (using 4 liters water volume), finishing with 2 hours postwashing 6 changes of tapwater. In the mutation of a high alkaloid *L. angustifolius* line, this method produced 16% (chlorophyll-related) chimeric M_1 plants, 29% M_2 families containing plants with chlorophyll mutations, and 34 single plants among 17,000 were selected as low alkaloid using Dragendorf reagent paper.

Mutation breeding will continue to provide genetic variation for lupin breeding programs, particularly where little natural variation is found in germplasm collections. Traits for improvement in current breeding programs using mutation include seed quality characteristics (e.g., seed coat reduction, increased seed protein, or changes to amino acid profile), herbicide and disease resistance, pod-wall reduction, and new sources of reduced pod shattering.

9.6 RESEARCH PRIORITIES

Some of the more imminent priorities should include development of a molecular map for all of the crop species of lupins. Progress toward this end is currently under way in some countries (e.g., Australia, Poland, England), and results should be forthcoming in the very near future. Lupin breeding programs would benefit from the incorporation of doubled haploid systems to accelerate the generation of new varieties and as an aid in molecular genetic studies. Sources of disease resistance to anthracnose is important for all species, and resistance to seed transmission of viruses is of high priority. Suggested research priorities for *Lupinus* crop improvement are listed below:

- Anthracnose, fusarium and grey mold (Botrytis) resistance
- Genome mapping and molecular marker development
- Development of a system to produce double haploids
- Interspecific crossing to introgress desirable characteristics among species, embryo or ovule rescue and culture
- Aphid tolerance in *L. luteus*
- Herbicide tolerance
- Higher digestible energy in *L. angustifolius, L. albus, L. luteus*
- Functional protein research and exploitation for niche markets
- Germplasm utilization—core collections, molecular genetic diversity analyses
- Increased protein concentration in *L. angustifolius*
- CMV nd BYMV tolerance in *L. angustifolius, L. luteus* and *L. mutabilius*
- Development of rapid screening methods (e.g., NIR) for seed quality variation
- Further investigation of plant architecture types for reduced lodging, higher harvest index, reliable ripening, and high yield
- Enhanced nitrogen-fixing ability
- Reduced cadmium accumulation in *L luteus*
- Reduced pod shattering and shedding in *L. luteus* for Mediterranean environments

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CHAPTER 10
Mungbean [*Vigna radiata* (L.) Wilczek]

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10.1 INTRODUCTION

Thirty years ago, mungbean [*Vigna radiata* (L.) Wilczek] was considered with other Asian *Vigna* to be "slow runners" (Borlaug, 1973). The impressive yield gains of other crops at that time had not occurred for mungbean. Today, the same comment could be made. Despite the importance of mungbean in the nutrition of Asian people, it is becoming increasingly familiar, particularly as bean sprouts, in health-conscious diets of other regions, and its production area is increasing annually; research efforts on mungbean do not appear to reflect this situation.

Mungbean is used in soups *(dhal),* deep fried savory cakes, noodles, and as flour for bread and biscuits. It is used as a vegetable in the form of bean sprouts and green pods. One kilogram of dry seeds of mungbean can yield 6 to 8 kg of sprouts. Mungbean seeds are rather sweet, have high (22.9%) protein (Duke, 1981) that is easily digestible, and lack flatulence factors in contrast to some other legumes.

There have been a number of reviews of mungbean from different perspectives [genetics (Fery, 1980); species relationships (Dana and Karmakar, 1990); agronomy (Rachie and Roberts, 1974); evolution and genetic resources (Smartt,1990); genetics, cytogenetics and breeding (Shanmu-gasundaram, 1988); *in vitro* techniques (Jaiwal and Gulati, 1995)]. In addition, several international symposia have been held on the topic of mungbean (AVRDC, 1988; Cowell, 1978; Srinives et al., 1996) and one book written on the crop (Poehlman, 1991). The objectives of this chapter are to discuss genetic resources, chromosome engineering, and crop improvement of mungbean in the light of information published since these reviews. In recent years, there has been progress in mungbean research, particularly in the field of genome analysis.

10.2 PRODUCTION

The area of mungbean production has been increasing at the rate of about 2.5% annually (Yang, 1996). A reason for this is that mungbean is a short-duration (60 to 90 days) crop that can easily be fitted into many different cropping systems (Poehlman, 1991). In India,

a large area is planted to a summer crop of mungbean between the winter crop of wheat and the monsoon season crops (Jain and Mehra, 1980). Worldwide production was estimated at 2.5 to 3 million t harvested from 5 million ha in 1991 (Poehlman, 1991). Recent estimates of dry bean production in India are 7 million t (2001/2002), but this figure includes *Phaseolus* species and other *Vigna* species (http://www.grainlegumes.com/). Since no separate production figures are given for mungbean, precise production figures cannot be given. However, the increasing importance of mungbean is indicated by plans to expand production of MYMV (mungbean yellow mosaic virus)-resistant early maturing lines to 1 million additional ha in the Indo-Gangetic plains by 2005 (AVRDC, 2001). In China, mungbean is grown on 1 million ha and is the most important *Vigna* species grown there (Zong, personal communication, 2003).

10.3 TAXONOMIC POSITION AND GENE POOLS OF MUNGBEAN

Mungbean belongs to a group of agriculturally important, warm weather and tropical legumes that includes *Phaseolus* cultigens, soybean, and other *Vigna* cultigens (Lackey, 1981). All these cultigens are in the same subtribe Phaseolinae (Kajita et al., 2001; Polhill and Raven, 1981). The *Vigna* is very closely related to *Phaseolus*, and the neotropical subgenus of *Vigna*, subgenus *Sigmoidotropis*, is a link between the two genera (Goel et al., 2002). The taxonomic differences between *Vigna* and *Phaseolus* have been clarified (Maréchal et al., 1978; Verdcourt, 1970). The close link between *Vigna* and *Phaseolus* has enabled comparative genome studies between these genera (discussed in section 10.6.3). Recently, a monograph has been published (Tomooka et al., 2002a) describing the 21 species in the subgenus *Ceratotropis*, genus *Vigna*, including four recently described new species (Tateishi and Maxted, 2002; Tomooka et al., 2002b). We use the nomenclature of this monograph here (Table 10.1).

Within the pan-tropical genus *Vigna*, *V. radiata* is within the subgenus *Ceratotropis*, which consists of a group of 21 species, of which 8 are cultivated (Tomooka et al., 2002). The place of *V. radiata* within the subgenus *Ceratotropis* has been clarified by molecular analyses and taxonomic studies (Doi et al., 2002; Tomooka et al., 2002c). These studies clearly align *V. radiata* with a small group of species, *V. mungo*, *V. grandiflora*, and *V. subramaniana*, with their diversity centered on South Asia, but also occurring in Southeast Asia. All these species share several morphological traits, such as epigeal germination and first and second leaves being narrowly elliptic to ovate and lacking a petiole. Tomooka et al. (2002a) gave a separate section name to this group of species in the subgenus *Ceratotropis*, section *Ceratotropis*.

Due to the close relationship between mungbean and black gram *(V. mungo)*, there was confusion surrounding their presumed progenitors, both of which occur in India and superficially have morphological similarities. However, broader stipules, pale yellow flowers, more ovules per pod, spreading pod with short brown hairs, and nonarillate hilum, as well as chemical and molecular characters, distinguish cultivated and wild *V. radiata* from cultivated and wild black gram (Chandel et al., 1984; Niyomdham, 1991).

Table 10.1 The Systematic Position of *Vigna radiata*

Family	Leguminosae
Subfamily	Papilionoideae
Tribe	Phaseoleae
Subtribe	Phaseolinae
Genus	*Vigna*
Subgenus	*Ceratotropis*
Section	*Ceratotropis*
Species	*Vigna radiata*
Variety	*Vigna radiata* var. *radiata* (cultigen)
	Vigna radiata var. *sublobata* (wild)

Vigna radiata var. *sublobata* has been confused with several other species. Niyomdham (1991) treated *V. grandiflora* as a variety of *V. radiata* called var. *grandiflorus*. However, following the proposal of Tateishi and Maxted (2002), this is now considered a distinct species. Distribution of this species is restricted to continental Southeast Asia, Thailand, and Cambodia. *Vigna subramaniana* has similar morphological characters to *V. radiata* var. *sublobata*, with which it has been confused. However, the distribution of *V. subramaniana* is confined to India and has smaller flowers and seeds, and seeds have irregular seedcoat reticulation (Tomooka et al., 2002). Another species that has been confused with *V. radiata* var. *sublobata* is *V. trinervia. Vigna trinervia* belongs to the section *Angulares*, and while morphological traits—especially in its vegetative parts—are similar to var. *sublobata, V. trinervia* can be distinguished by its long peduncle covered with brown hispid hairs and bright yellow flower (Tomooka et al., 2002a).

The gene pools of *Vigna radiata*, following the system of Harlan and de Wet (1971), are shown (Figure 10.1).

10.4 DIVERSITY OF THE PRIMARY GENE POOL AND ORIGIN *VIGNA RADIATA*

Vigna radiata var. *sublobata*, the wild progenitor of mungbean, is widely distributed across tropical Africa, Oman in western Asia, South and Southeast Asia to Papua New Guinea and Australia (Figure 10.2). India is considered the center of diversity of the wild form (Lawn, 1995). Weedy forms of mungbean are also reported from India (Paroda and Thomas, 1988). In India, wild mungbean (var. *sublobata*) is found in hilly tracks, particularly in the northern *terai* region and Western Ghats (Arora and Nayar, 1984; Saravanakumar et al., 2003). Artificial crossing between *V. radiata* var. *radiata* and var.

sublobata is not difficult (Egawa, 1988; James et al., 1999; Miyazaki, 1982), so natural crossing where cultivated and wild mungbean are sympatric might be expected.

Various studies have revealed considerable variation in the wild progenitor of mungbean. Intraspecific hybrids between *V. radiata* and *V. radiata* var. *sublobata* from different regions result in F_1 hybrids with different levels of pollen fertility (Miyazaki, 1982). Linkage maps derived from crosses involving accessions of *V. radiata* var. *sublobata* from different geographic locations with cultivated *V. radiata* reveal different levels of distortion in segregating generations (see section 10.6.2.1; Gulati and Jaiwal, 1994; Lambrides and Imrie, 1999). Molecular analyses (AFLP and RAPD) have revealed that accessions of *V. radiata* var. *sublobata* from different geographic regions are genetically differentiated, especially Australian accessions (Saravanakumar et al., 2004). The long-time presence of var. *sublo-*

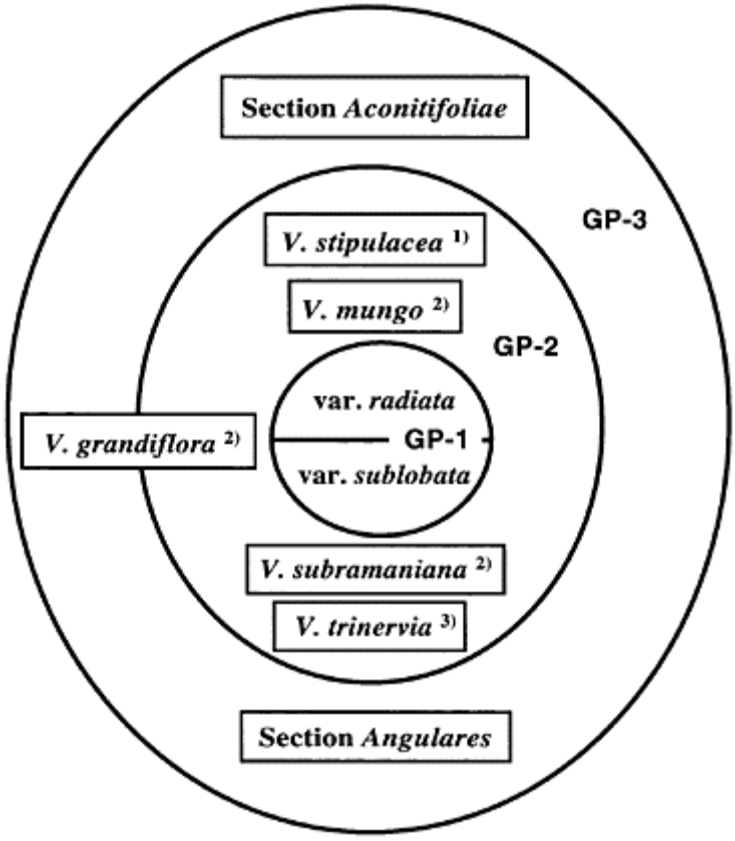

Figure 10.1 Gene pools of mungbean *(Vigna radiata):* Gene pool 1 (GP1) constitutes the biological species; Gene pool 2 (GP2) includes those species

that cross with GP-1 with at least some fertility; Gene pool 3 (GP3) includes those species where gene transfer requires radical techniques. Most of the species in sections *Aconitifoliae* and *Angulares* have not been adequately studied to know their cross compatibility with mungbean. [1]This species is in section *Aconitifoliae;* [2]these species are in section *Ceratotropis*.

bata in Australia is indicated by its ecotypic differentiation and use as an aboriginal food (Lawn and Cottrell, 1988; Lawn and Watkinson, 2002). The distribution, ecology, and plant types of *V. radiata* var. *sublobata* in Australia and nearby countries have been described in detail (Lawn and Watkinson, 2002). A distinctive form with deeply lobed leaflets is reported from the clay soils of Central Queensland. In addition, a short-lived perennial form with thick fleshy roots that has not been seen in other regions was found in northeastern tropical Australia. *Vigna radiata* var. *sublobata* in Australia has a twining, more gracile habit compared to this taxa from other regions. Also, Australian accessions of *V. radiata* var. *sublobata*, while varying in seed size and pod color, lack "weedy characteristics" such as green testa color and bushy plant type found in Asia (Lawn and Watkinson, 2002).

There have been many studies of genetic diversity in mainly Indian landraces of mungbean (Bisht et al., 1998; Lakhanpaul et al., 2000; Raje and Rao, 2001; Roshan et al., 1998; Santall et al.; Tomooka et al., 1991, 1992a; Venkatakrishna et al., 2000). The most comprehensive studies of landrace differentiation using germplasm covering much of the distribution of mungbean in Asia are based on protein banding and plant growth types (Tomooka et al., 1991; Tomooka et al., 1992a). Results support the view of Vavilov (1926) and pointed to Western Asia, Afghanistan, Iran, and Iraq, as being an important gene center for the crop, and India a likely place of origin. The presence of primitive forms and diverse protein-type genotypes for mungbean in Afghanistan, Iran, and Iraq may be the result of lack of selection there, since that seems to be the case with other crops in the region (Vavilov, 1926).

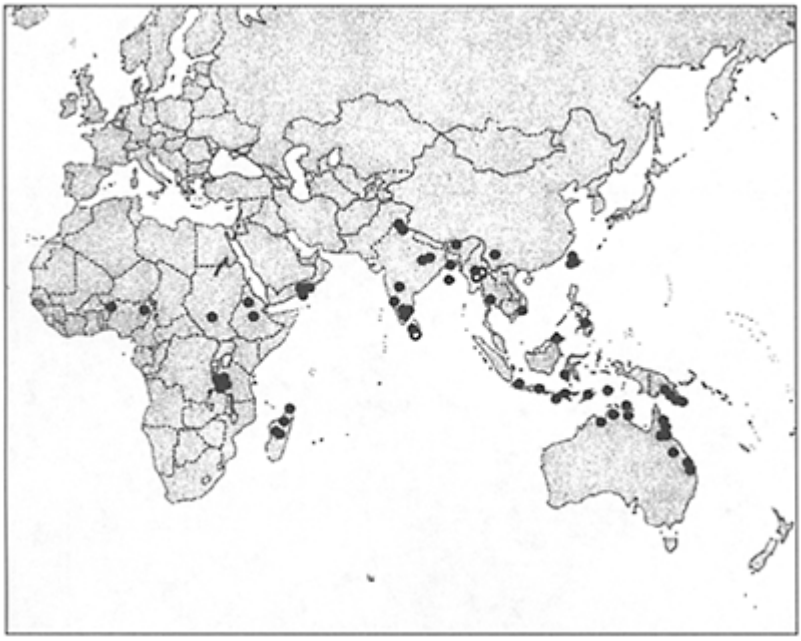

Figure 10.2 Distribution of *Vigna radiata* var. *sublobata* based on herbarium specimens and direct collection. From Tomooka, N., Vaughan, D.A., Moss, H., and Maxted, N.*The Asian Vigna.* Kluwer Academic Press, Dordrecht, The Netherlands. 2002. With permission.

Three lines of evidence support India as the probable area of mungbean domestication:

1. Existence of wild and weedy relatives of mungbean in India.
2. Preserved remains of *Vigna* seeds at archaeological sites in India. The earliest archaeological site where *Vigna* remains have been found is Navdatoli, Madhya Pradesh, and the remains date back to between 3500 and 3000 BC (Jain and Mehra, 1980). In Tamil Nadu *Vigna* remains date back to between 2500 and 3000 BC (Vishu Mittre, 1989).
3. Residual diversity of landraces across India.

Indian mungbean landraces have the most diverse protein types and growth types. India also has many populations of wild and weedy mungbean, and remains of mungbean at Indian archaeological sites have been found. Thus, India is where mungbean is thought to have been domesticated. From India, mungbean spread to the Afghanistan-Iran-Iraq region and Southeast Asia in early times. In the Afghanistan-Iran-Iraq region, mungbean

landraces are considered primitive (seeds are small and of various colors, often mixed, plants have many lateral branches), and they retain diverse protein types. In contrast, Southeast Asian mungbean landraces mainly have large shiny green seeds, plants are tall with thick main stems, are late maturing, and protein types are simple. In Southeast Asia, mungbean has probably been under high selection pressure from farmers. In East Asian countries, mungbean landraces include growth type diversity that is intermediate between Afghanistan-Iran-Iraq and Southeast Asian types. In addition, protein types that are not found in the southeast can be found in East Asia. Therefore, East Asian landraces may have come from Afghanistan-Iran-Iraq region along the Silk Road through Central Asia and western China, as well as Southeast Asia. The presumed routes along which mungbean spread to different parts of Asia, based on protein banding studies and plant habit, are shown in Figure 10.3.

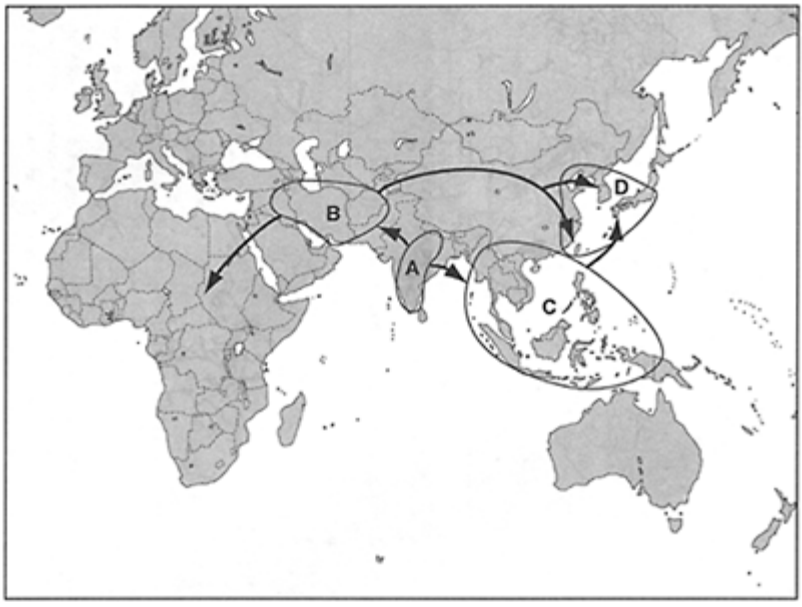

Figure 10.3 Origin and route of dispersal of mungbean deduced from the geographical distribution of habit and protein types. Indian (A) mungbean landraces have the most diverse protein types and growth types. In the Afghanistan-lran-lraq region (B), mungbean landraces are considered primitive. In contrast, Southeast Asian (C) mungbean

landraces mainly have large shiny green seeds, plants are tall with thick main stems and are late maturing; protein types are simple. In East Asian countries (D) mungbean landraces include growth type diversity that is intermediate between Afghanistan-Iran-Iraq and Southeast Asian types.

Table 10.2 Main Genebanks Conserving *Vigna radiata* and Their Holdings

Institution	No. Acc.*
Asian Vegetable Research and Development Center, Shanhua, Taiwan	5612(4)
Australian Plant Genetic Resources System, Australia	609(34)
Chai Nat Field Crops Research Center, Chai Nat, Thailand	936
PGRCU-USDA, Griffin, Georgia	3889(1)
Malang Research Institute for Food Crops (MARIF), Indonesia	930
Bangladesh Agricultural Research Institute, Joydebpur, Bangladesh	498
Institute of Crop Germplasm Resources, CAAS, Beijing	5217
National Botanic Garden, Meise, Belgium	13(17)
MAFF Genebank, National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan**	1068(14)
Instituto Colombiano Agropecuario (ICA), Palmira Valley, Colombia	135
Punjab Agricultural University (PAU), Ludhiana, India	3000
National Board for Plant Genetic Resources, New Dehli, India	2220
Central Research Institute for Food Crops (CRIFC), Sukamandi, Indonesia	2172
Plant Genetic Resources Institute, Islamabad, Pakistan	626
Inst. Plant Breeding, Uni. of the Philippines, Los Banos (IPB-UPLB), Philippines	5736
University of Missouri (UM), Columbia, Missouri, U.S.	2100

* Number of accessions for wild form shown in parenthesis.
** Refers only to the active collection.

10.5 GERMPLASM RESOURCES

The main genebanks that have accessions of cultivated and wild mungbean are listed (Table 10.2). The largest collection is conserved at the Asian Vegetable Research and Development Center (AVRDC). AVRDC has mungbean as one of its mandate crops. Consequently, it has been active in collaboration with various countries in mungbean improvement, and a network approach is being used to evaluate germplasm. Currently, a major focus of this network approach is to produce high-yielding varieties with stable resistance to mungbean yellow mosaic virus (AVRDC, 2000).

Characterization and evaluation descriptors for mungbean genetic resources have been developed (IBPGR, 1980). A catalogue of mungbean genetic resources and listing of mungbean varieties released worldwide have been published (Shanmugasundaram, 1988; Tsay et al., 1989).

10.6 CYTOGENETICS AND GENOMICS

10.6.1 Chromosome Studies

Subsequent to determining the chromosome number of mungbean (2n=2x=22) (Karpechenko, 1925), cytological studies have been limited to studies of the size and shape of *V. radiata* chromosomes (Table 10.3) (Bhatnagar, 1974; Joseph and Boukamp, 1978; Krishnan and De, 1965; Shrivastava et al., 1973; Venora et al., 1999). The nuclear DNA content of *V. radiata* has been calculated as 579 Mbp/1C (Arumganathan and Earle, 1991). The DNA quantity in individual chromosomes has been estimated (Table 10.3) (Parida et al., 1990).

Studies of chromosome size in *V. radiata* are useful in relation to development of linkage maps. The most recent estimates for chromosome size and chromosome DNA amount suggest the smallest chromosomes in the mungbean haploid genome are about half the size of the largest (Table 10.3). Hence, variation in well-saturated linkage maps would be expected to show a similar variation in linkage group map distances.

Table 10.3 Information on the Chromosomes of *Vigna radiata**

Chro mosome No.	Relative Length %	Chro mosome Length (µm)	Long Arm (µm)	Short Arm (µm)	Satellite (µm)	Arm Ratio Long /Short	Chro mosome T ype	DNA Amount (pg)
1	13.60	2.10aA	0.95±0.15	0.65±0.01	0.05±0.09	1.21dC	metacentric	0.331
2	11.15	1.77bB	0.72±0.08	0.61 ± 0.05	0.044±0.07	1.89aA	submetacentric	0.321
3	10.50	1.62cC	1.00±0.10	0.62±0.05	—	1.63cB	metacentric	0.289
4	9.91	1.53dD	0.84±0.08	0.69±0.11	—	1.22dC	metacentric	0.265
5	9.10	1.40eE	0.77±0.08	0.63±0.06	—	1.23dC	metacentric	0.258
6	8.73	1.35fE	0.75±0.06	0.60±0.08	—	1.24dC	metacentric	0.245
7	8.23	1.27gF	0.70±0.07	0.57±0.04	—	1.22dC	metacentric	0.216
8	7.77	1.20hG	0.76±0.10	0.44±0.06	—	1.72bB	submetacentric	0.206
9	7.47	1.15iG	0.63±0.07	0.52±0.07	—	1.22dC	metacentric	0.197
10	6.98	1.08IH	0.58±0.05	0.50±0.04	—	1.17dC	metacentric	0.175
11	6.23	0.96ml	0.52±0.08	0.44±0.03	—	1.19dC	metacentric	0.166

* Data from Venora et al., except DNA amount from Parida et al. (1990).

Table 10.4 Linkage Maps Developed for *Vigna radiata*

Cross Combination	Population (Plants/Lines) Analyzed	Markers Used	Linkage Groups Resolved	Map Distance	Level of Distortion	Reference
V. *radiata* var. *radiata* × V. *radiata* var. *sublobata* (from Madagascar)	F_2 population (58)	151 RFLP, 20 cDNA and 1 pest locus	14	1570cM	12%	Menacio-Hautea et al., 1992
V. *radiata* var. *radiata* × V. *radiata* var. *sublobata* (from Australia)	F2 population (67)	52 RFLP, 56 RAPD, 2 morphological markers	12	758.3cM	14.5%	Lambrides et al., 2000
V. *radiata* var.	Recombinant	113 RAPD, 2	12	691.7cM	24%	Lambrides

radiata × V. *radiata* var. *sublobata* (from Australia)	inbreed (67)	morphological markers				et al., 2000
V. *radiata* (cv. Berken) × V. *radiata* var. *sublobata* (from Australia ACC41)	Recombinant inbreed (80)	255 RFLP markers	13	737.9cM	30.8%	Humphrey et al., 2002

10.6.2 Linkage Maps

10.6.2.1 Nuclear Genome

The genome linkage maps for mungbean have so far been based primarily RFLP probes and RAPD markers (Table 10.4; Figure 10.4). However, the most saturated linkage maps for *Vigna* species, based on crosses involving *V. angularis* (Kaga et al., 2003) (see Chapter 11) and *V. unguiculata* (Ouédraogo et al., 2002) (see Chapter 5), have both incorporated many AFLP markers to help saturate the map. Thus, incorporation of AFLP markers into the mungbean linkage map would be expected to resolve the 11 linkage groups, but this has yet to be achieved. In addition, the location of simple sequence repeats (SSR or microsatellite) markers on linkage maps of *Vigna* cultigens have not been reported. However, recently SSR markers have been found in *V. radiata*, The first report of microsatellites in *V. radiata* was based on a database search and found 6 SSR sequences based on a search of 67.1 kb long sequence, and of these SSR sequences, two were the same (Yu et al., 1999). Using a library-enrichment procedure (5′ anchored PCR technique), 23 dinucleotide, 15 tetranucleotide microsatellites, and 6 cryptic SSR have been reported (Kumar et al., 2002a, 2002b). These SSR markers, and more extensive libraries from related *Vigna* species (Wang et al., 2004), should be useful for improving the linkage map of mungbean and in gene mapping.

To date, there have been four linkage maps published for *V. radiata* (Table 10.4) (Humphry et al., 2002; Lambrides et al., 2000; Menacio-Hautea et al., 1992). A comparison among these maps is shown (Table 10.4). None of the maps reported so far have resolved the 11 linkage groups, equivalent to the haploid chromosome number of *V. radiata*. To overcome the limited number of marker clones for *V. radiata*, many DNA marker clones from related species, such as *Glycine max*, *P. vulgaris*, and *V. unguiculata*, have been used to increase the saturation of the genome maps of *V. radiata* (Boutin et al., 1995; Lambrides et al., 2000; Menacio-Hautea et al., 1993; Chaitieng et al., 2002).

The genome maps for *V. radiata* have been based on crosses between *V. radiata* var. *radiata* and *V. radiata* var. *sublobata.* In one genome map, the *V. radiata* var. *sublobata* accession came from Madagascar (TC1966) (Menacio-Hautea et al., 1993); in the others the var. *sublobata* accessions came from Australia (Lambrides et al., 2000). In the resulting genome maps, while the order of genome markers was similar, the level of distortion was higher in the cross that involved Australian accessions of var. *sublobata*,

and regions of distortion did not coincide with those produced with the accession from Madagascar. This suggests that var. *sublobata* has considerable intraspecific genetic diversity and that the Australian form of var. *sublobata* is more distantly related to cultivated *V. radiata* than this variety from Madagascar.

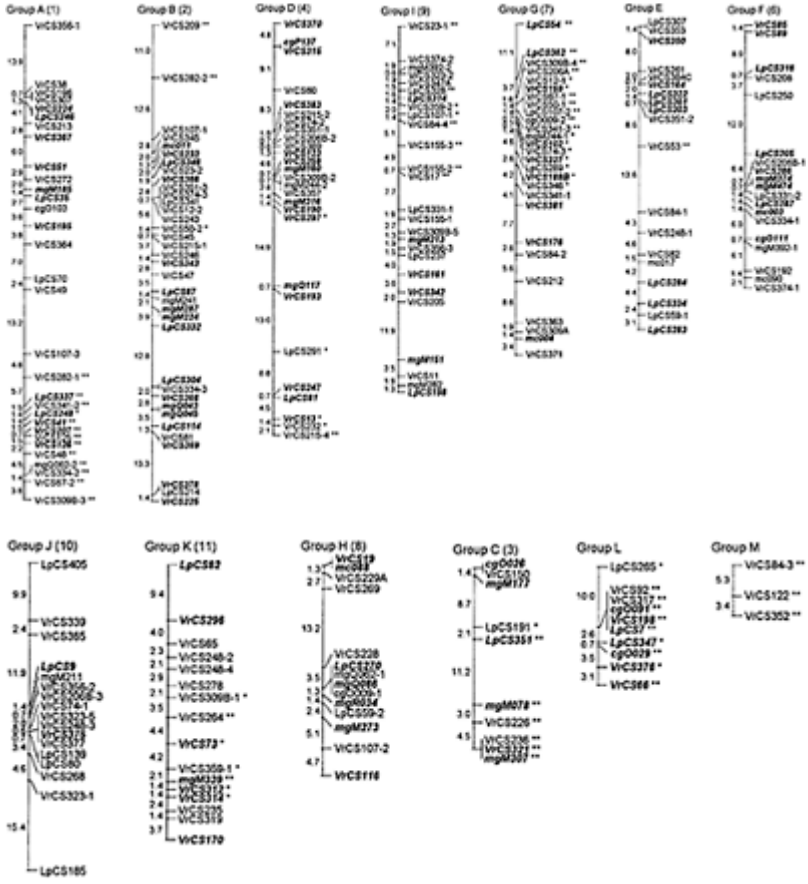

Figure 10.4 Linkage map of mungbean *(Vigna radiata)* constructed with RFLP markers. Linkage groups are ordered by size; letters at the top of linkage group correspond to linkage group of Humphry et al.; number in brackets indicates previous order of linkage groups. The long vertical bars indicate linkage groups, and horizontal bars indicate locus position. Genetic

distance (cM) and locus names are indicated to the left and right of linkage groups, respectively. From Humphry, M.E., Konduri, V., Lambrides, C.J., Magner, T., Mclntyre, C.L., Aitken, E.A.B., Liu, C.J., *Theor. Appl. Genet.*. 105, 160. 2002. With permission of Springer-Verlag and the corresponding author.

Table 10.5 Status of Transformation to Produce Plants in *Vigna radiata*

Transformed Plants Produced*	Method for Gene Transfer	Variety	Explant Used	Reference
*uidA a*nd nptll genes	*Agrobacterium*	B1, T44	Cotyledons	Pal et al., 1991
uidA and *npt*ll genes	Particle bombardment	ML-5, K-851	Ungerminated immature embryos	Bhargava and Smigocki, 1994
uidA, *nptll* and hpt genes	Agrobacterium	K-851	Cotyledon nodes	Jaiwal et al., 1987

* *uidA*-β-glucuronidase; *npt*ll-neomycin phosphotransferase; *hpt*-hygromycin phospho-transferase.

10.6.2.2 Mitochondrial Genome

A 7.1 kb region of the mungbean mitochondrial genome has been sequenced covering the *orf140-nad3-rps12-atp1* gene cluster that occurs as a single copy (Cheng and Dai, 2000). A high level of conservation was observed in coding region sequences of *nad3, rps12,* and *atp1* with other plant species. Homology was found in the 3′ flanking region of *atp 1* gene with soybean and common bean that is specific to this group of legumes (Cheng and Dai, 2000).

10.6.2.3 Chloroplast Genome

The mungbean chloroplast measures 150 kb in length, and it includes two identical sequences of 23 kb that contain the ribosomal genes and are arranged as an inverted repeat separated by single-copy regions of 21 and 83 kb (Palmer and Thompson, 1981). Within the single-copy region of 83 kb is a 50 kb inversion that is found in all legumes (Palmer, 1985). Thirty-six protein genes in the mungbean chloroplast genome have been mapped, and, by comparison with common bean and soybean, it was found that most of the single copy region (78 kb segment) of the mungbean and common bean cpDNA is inverted relative to soybean. This indicates that this 78 kb segment inversion occurred in the common ancestor of mungbean and common bean (both in subtribe Phaseolinae of

tribe Phaseoleae) after divergence, from the lineage leading to soybean (subtribe Glycininae of tribe Phaseoleae) (Palmer et al., 1988).

10.6.3 Comparative Genomics

10.6.3.1 Mungbean Compared with Cowpea, Common Bean, and Soybean

There is considerable scope for understanding genome organization in *V. radiata* by using probes from and comparison with better developed genome maps in other related species. Comparisons of genome maps of *V. radiata* with *V. unguiculata* and *P. vulgaris* have revealed conserved blocks of considerable size, with some containing loci for important traits (Fatokun et al., 1992; Maughan et al., 1996; Menacio-Hautea et al., 1993). For example, the major QTL for seed weight in cowpea and mungbean span the same RFLP markers (Fatokun et al., 1992).

One of the best developed genome maps among legumes is that of the soybean. Comparison of *V. radiata* and *G. max* revealed a different type of genome organization than the comparison of *G. max* with *P. vulgaris*. Conserved linkage blocks between *G. max* and *V. radiata* are smaller and are highly scattered (Boutin et al., 1995). Comparative genomic studies have also revealed that *V. radiata* genome is more rearranged than *Phaseolus* in relation to soybean (Kim et al., 2002).

10.6.3.2 Mungbean Compared with Other Subgenus Ceratotropis Species

Dana and Karmakar (1990) proposed two genome groups for the subgenus *Ceratotropis*, AA and A_1A_1, based on crossability, hybrid fertility, and chromosome pairing. The genome group AA includes mungbean and *Vigna aconitifolia, V. dalzelliana, V. khandalensis, V. mungo,* and *V. trilobata*, while A_1A_1 consists of *V. angularis* and *V. umbellata*. There have been many reports of interspecific hybridization involving mungbean (for reviews see Dana and Karmakar, 1990; Tomooka et al. 2002a). Results of interspecific hybridization suggests that mungbean and related Asian *Vigna* have more complexity in genome structure than indicated by just two groups for the 21 species in the subgenus *Ceratotropis*. A complete study and synthesis of information of genomes in the subgenus *Ceratotropis* have yet to be undertaken.

Studies of the ribosomal DNA spacer of mungbean have revealed that mungbean has a 174 bp rDNA subrepeat structure downstream from the 25S rRNA coding region (Gerstner et al., 1988; Schiebel et al., 1989; Unfried et al., 1991). These subrepeat sequences also occur as prominent independent satellite DNA clustered at several positions within the genome of mungbean but not in azuki bean. In azuki bean, the 174 bp repeats were found only in the intergenic spacer of rDNA (Unfried et al., 1991). Sequence homology within eight randomly cloned rDNA subrepeats of *Vigna angularis* ranged from 91 to 99% but varied 81 to 87% when rDNA repeats of *V. radiata* and *V. angularis* were compared (Unfried et al., 1991).

10.7 TISSUE CULTURE, REGENERATION, AND SOMACLONAL VARIATION

There have been several reports of successful transformation of mungbean resulting in plants (Table 10.5) (Nagl et al., 1997; Somers et al., 2003). These have used two methods to introduce genes, particle bombardment, and *Agrobacterium*. However, transformation leading to plants is only possible using plant tissues. For the full potential of *in vitro* transformation systems to be realized in mungbean regeneration from cell suspensions, callus and protoplast cultures are needed, and such systems have not yet been developed (Kaga et al., 2004).

Systems have been developed to regenerate plants of *Vigna radiata* from various tissues including deapexed embryo, excised shoot tips, cotyledons, and cotyledon nodes (Goel et al., 1983; Gulati and Jaiwal, 1990, 1992, 1994; Mathews, 1987). Differences in regeneration success reflect explant used, its physiological status, genotype, and culture conditions. Differences have been observed between the two cotyledons of mungbean in relation to regeneration (Chandra and Pal, 1996). Shoot regeneration and regeneration efficiency were higher when the cotyledon most closely attached to the embryo was used (Chandra and Pal, 1996).

Adventitious shoots from cotyledon explants have been used in selection systems to generate salt-resistant lines (Gulati and Jaiwal, 1993). Stable salt-resistant shoots were obtained, and plants were obtained from those shoots.

10.8 GERMPLASM ENHANCEMENT

While mungbean is a predominantly self-pollinated crop, cleistogamy of up to 42% has been reported (Rachie and Roberts, 1974). Natural cross-pollination has been reported as 0.5 to 3% (Empig et al., 1970; van Rheeman, 1964). Hybridization procedures for *Vigna* species have been described (Tomooka et al., 2002a).

Table 10.6 Traits That May Be of Use in Breeding Reported from *Vigna radiata* var. *sublobata*

Trait	Reference
Hard seededness (to prevent premature sprouting)	Lawn and Cottrell, 1998
Perennial tendency	Lawn and Cottrell, 1998
Tolerance to saline soils	Lawn and Cottrell, 1998
Tolerance to alkaline soils	Lawn and Cottrell, 1998
Tolerance to cool temperatures	Lawn and Cottrell, 1998
Possible source of drought resistance	Ignacimuthu and Babu, 1987
High rate of photosynthesis	Ignacimuthu and Babu, 1987

High protein content	Ignacimuthu and Babu,1987; Lukoki et al., 1980
Good amino acid composition	Ignacimuthu and Babu, 1987; Lukoki et al., 1980
Bruchid resistance	Fujii and Myazaki, 1987
Genetic bridge in crossing *Vigna* spp.	Miyazaki, 1982

10.8.1 Conventional Breeding

The principle of conventional mungbean breeding is reviewed by Srinives (1996) and objectives can be listed as follows:

1. High yield, uniform maturity, and adaptation to various cropping seasons
2. Resistance to *Cercospora* leaf spot, powdery mildew, mungbean yellow mosaic virus (MYMV)
3. Resistance to bruchids, bean flies, and mungbean pod borers
4. Breeding for high tolerance to alkaline soils
5. Improved nutrient content, such as increasing sulfur-containing amino acids of protein and altering seed starch composition for the use of mungbean in different foods

For some of these traits, sources of germplasm have been identified or improved lines have been produced. Selected useful germplasm includes:

Bruchid resistance—TC1966 wild mungbean, var. *sublobata* (Fujii and Miyazaki, 1987)

Mungbean Yellow Mosaic Virus (MYMV)—ML267, ML613, NM92, VC6372 (45–8–1), NM94, and VC6368 (46–40–4) were consistently MYMV-resistant/tolerant across locations (AVRDC, 2000)

Powdery mildew (*Erysiphe polygoni* DC.)—ML3, ML5, VC3890A, VC1210A (Chaitieng et al., 2002; Reddy et al., 1994; Young et al, 1993)

Nickel tolerance—Dhauli, PDM-116 (Samantaray et al., 1998)

White fly *(Bemisia tabaci)* that spreads MYMV—ML537, ML370 (Chhabra and Kooner, 1993)

Thrips *(Megalurothrips distalis)*—SML12, SML103 (Chhabra and Kooner, 1994).

In addition to useful germplasm in cultivated mungbean, potentially useful traits have been reported to be present in accessions of *V. radiata* var. *sublobata*, which might be of use in mungbean breeding (Table 10.6).

10.8.2 Gene Mapping

10.8.2.1 Bruchid Resistance

One of the most destructive pests of mungbean are bruchid beetles that can do much damage to mungbean, particularly in storage. The most important of these species are *Callosobruchus chinensis* and *C. maculatus*. Two cultivated lines with moderate to high levels of resistance to *C. chinensis* have been reported (Talekar and Lin, 1992). In addition, many sources of resistance have been found in wild relatives of mungbean and some other cultivated *Vigna* (Kashiwaba et al., 2003; Lambrides et al., 2000; Tomooka et al., 2000). One of the first sources of resistance to bruchids in Asian *Vigna* was found in an accession of *V. radiata* var. *sublobata* (TC1966) from Madagascar (Fujii and Miyazaki, 1987). This accession has been the focus of many studies and also used in breeding (Fujii et al., 1989; Kaga and Ishimoto, 1998; Tomooka et al., 1992b; Young et al., 1992). The first attempt to map bruchid resistance located the gene to within 3.6cM of an RFLP marker (Young et al., 1992). Subsequent efforts were made to map this bruchid resistance gene, because it was closely associated with novel cyclopeptide alkaloids and inhibitory activity against bean bug (*Riptortus clavatus* Thunberg). The gene for resistance to bruchids from TC1966 has now been located to within 0.2cM of RFLP markers, and this may enable the gene to be cloned within a large genomic library (Kaga and Ishimoto, 1998). Recently, a small cysteine-rich protein, belonging to the plant defensin family, has been isolated from mungbean breeding lines with TC1966 bruchid resistance gene (Chen et al., 2002). While this protein has been found to have a lethal effect on *C. chinensis* in an artifical seed-feeding test, it was not considered to be the basis for bruchid resistance in TC1966 (Chen et al., 2002). The basis for bruchid resistance in TC1966 remains unknown.

10.8.2.2 Powdery Mildew

Powdery mildew is a major disease of mungbean, and breeding for resistance to this disease is a priority. However, breeding progress has been slow because there appear to be many races of the disease, though these have not yet been described. Several sources of resistance or partial resistance to the disease have been identified (Chaitieng et al., 2002; Reddy et al., 1994). Attempts have been made to map resistance in two of these resistance sources VC3890A (an AVRDC line released as Tainan 4 in Taiwan in 1989) and VC1210A (a cross between ML-3 from India and CES 87–17 from the Philippines). RFLP analysis of a mapping population developed using VC3890A revealed three genome regions on different linkage groups having an effect on powdery mildew response that explained 58% of the total variation (Young et al., 1993). Chaitieng et al. (2002) analyzed a mapping population using VC1210A as the resistant parent and found a QTL for field resistance to Thai races of powdery mildew that explained 65% of the variation to powdery mildew resistance.

10.9 FUTURE PERSPECTIVES

1. There is yet to be a comprehensive analysis of *V. radiata* var. *sublobata* germplasm across its entire range. This reflects both lack of specific collecting of *V. radiata* in some areas, such as Africa, and also a lack of exchange of germplasm so that worldwide representation can be assembled and studied.
2. Studies of weedy mungbean are lacking. Their extent and characteristics have not been described.
3. The genetic linkage map of *V. radiata* remains less well developed than that for *V. angularis* (see Chapter 11) and *V. unguiculata* (see Chapter 5). Despite a *V. radiata* genetic linkage map being developed in the early 1990s, efforts to improve this map were not carried forward as they were with the *V. unguiculata* linkage map. Instead, separate mapping populations have been developed and studied.
4. Efforts to develop a universal intron-targeted PCR-based marker set for the legume family for comparative legume map, using *Medicago truncatula* ESTs and *Arabidopsis* as reference genomes, will help in understanding synteny across economically important legumes, including mungbean (Lee et al., 2001; Wang et al., 2003). The next few years will likely see the mungbean genome map improved and compared with other legumes. However, further efforts to generate markers specifically for mungbean are important so that molecular approaches to breeding in mungbean are not impeded. It is perhaps not where mungbean shares commonality of its genome with other legumes that may be important, but where it differs.
5. In order to promote consistency and uniformity in genetics and genomics, an international *Vigna* genetics committee, perhaps in collaboration with scientists working on *Phaseolus*, needs to be established. Such a committee would give a stimulus to *Vigna* research, and such committees have been very valuable for other crops.

10.10 USEFUL WEB SITES

For bibliographic information on mungbean from AVRDC library:
http://avrdc.org/
For recent information on legume biotechnology:
http://beangenes.cws.ndsu.nodak.edu/
http://www.ncbi.nlm.nih.gov//Taxonomy
Note: All authors contributed equally to this chapter. This review was completed at the beginning of 2003.

10.11 SUMMARY

Mungbean [*Vigna radiata* (L.) Wilczek], a short-duration crop, has high quality protein that is easily digested, and its production is increasing annually, particularly in Asia, its center of diversity. While several mungbean nuclear genome maps have been developed, none have yet resolved the 11 linkage groups of this crop. The chloroplast genome of

mungbean has been mapped, and parts of the mitochondria genome have been sequenced. Comparative genomics of mungbean with other closely related legumes, cowpea [*V. unguiculata* (L.) Walpers], common bean (*Phaseolus vulgaris* L.), and soybean [*Glycine max* (L.) Merr.], have been conducted. Mungbean regeneration from cell suspensions, callus, and protoplast cultures have not yet been developed, but have been developed for various tissues such as the cotyledons and cotyledon nodes. Breeding efforts have focused on resistance to virus diseases, bruchid beetles (*Callosobruchus* spp.), and powdery mildew (*Erysiphe polygoni* DC.). A great deal of genetic resources, cytological, genetic, genomic, and tissue culture research is needed to bring our knowledge base of this important crop to the level of other major legumes.

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CHAPTER 11
Azuki Bean [*Vigna angularis* (Willd.) Ohwi & Ohashi

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11.1 INTRODUCTION

The Chinese characters for azuki bean [Vigna angularis (Willd.) Ohwi & Ohashi], 小豆, means "small bean," while the kanji characters for soybean, 大豆, mean "big bean." These characters also reflect the relative importance of these two crops in East Asia, where they are the main legumes in people's diets.

Despite being a minor crop, azuki bean has considerable cultural importance in East Asia. In Japan, azuki bean is associated with success and good fortune. Thus, to celebrate happy occasions such as a births or weddings, or success in an exam, glutinous rice is cooked with azuki bean to produce "sekihan" or red rice. Azuki bean is an important component of traditional Japanese foods such as steam "an" (azuki paste) buns; azuki paste mixed with agar-agar makes a jellylike dessert (yokan); taiyaki is a waffle filled with azuki paste. Azuki flour mixed with wheat flour is used to make noodles in parts of China. In traditional Chinese medicine, azuki has been reported to treat many ailments (Sacks, 1977). In Nepal, young pods are used as a vegetable (Baral, personal communication, 2002). Azuki beans contain about 21% protein (Duke, 1981).

Azuki bean is a crop that has received attention at national and regional levels in China, Japan, and Korea. It is a crop that has not been the subject of international symposia nor the focus of regional networks. Much of the literature on azuki bean, particularly related to production, breeding, and evaluation, is not in the international literature. A detailed account of the botany, production, and use of azuki bean was written by Lumpkin and McClary (1994). The objective of this chapter is to focus on recent information related to azuki bean.

11.2 PRODUCTION

The worldwide production of azuki bean is difficult to estimate, since statistics for azuki bean are usually combined with other minor legumes. Accurate figures are available for

Japan where 70,000 to 90,000 metric tons were produced annually between 1996 and 2000. Most of Japanese production (80 to 85%) is on the northern island of Hokkaido, where wild azuki is not found. In 2000, Japan imported 30,495 metric tons of azuki bean from the world's main producer, China (26,508 t), and the U.S. (2,393 t). In China, azuki bean is cultivated on 470,000 ha (Zong, personal communication, 2003), which would suggest that China may have an annual production of about 700,000 metric tons.

Other traditional areas where azuki beans are grown are the Korean peninsula, Nepal, and Bhutan. Countries that have investigated azuki bean as a specialist crop are Australia, New Zealand, and the U.S. These countries have viewed azuki as a crop to export to Japan. Recently, Australia and China have conducted collaborative research on azuki bean production (Wang et al., 2001).

11.3 DISTRIBUTION OF WILD AND WEEDY AZUKI

The wild progenitor of azuki bean is *V. angularis* var. *nipponensis*. It grows naturally from northern East Asia to northern South Asia (Figure 11.1). *Vigna angularis* var. *nipponensis* is recorded from China, including Taiwan; India; Japan; Korea; (North and South); Myanmar; and Nepal. Unfortunately, little information is available on *V. angularis* var. *nipponensis* in China, and Tomooka et al. (2002a) only records four herbarium specimens from China. Apparently *V. angularis* var. *nipponensis* is present in various parts of China, such as Yunnan Province and the mountains of Hubei province, but specific and systematic collecting of wild *Vigna* genetic resources throughout China has not been conducted (Zong, personal communication, 2002.). At southern latitudes, *V. angularis* var. *nipponensis* is found at higher altitude. It occurs in the mountains of Taiwan, China, but is not present on low-lying islands of Okinawa prefecture, Japan. Recently, taxa of the *V. angularis* complex have been found in the Chin Hills of Myanmar between 1200 and 1800 m elevation (Tomooka et al., 2003). In Nepal, a species closely related to *V. angularis* but distributed at lower elevations has recently been described as *V. nepalensis* (Tateishi and Maxted, 2002). In Korea, *V. angularis* var. *nipponensis* and *V. nakashimae* can be found sometimes sympatric (Yoon, personal communication, 1999).

11.4 GENETIC RESOURCES

The main genebanks conserving azuki genetic resources and their holdings are shown (Table 11.1). The conserved germplasm of cultivated azuki beans is quite comprehensive over most of its range, except for the Himalayan region and, possibly, southern China. However, systematic collections for the wild relatives of azuki have only been conducted in a few areas. Germplasm in genebanks of *V. angularis* var. *nipponensis* is poorly represented from areas of lower latitude such as China and the Himalayan foothills. Wild *Vigna* species are often sympatric (Tomooka et al., 2002a). Consequently, without careful attention, different species at the same site may not be recognized. There is also little information about weedy azuki outside Japan, where it was first recognized (Yamaguchi, 1992).

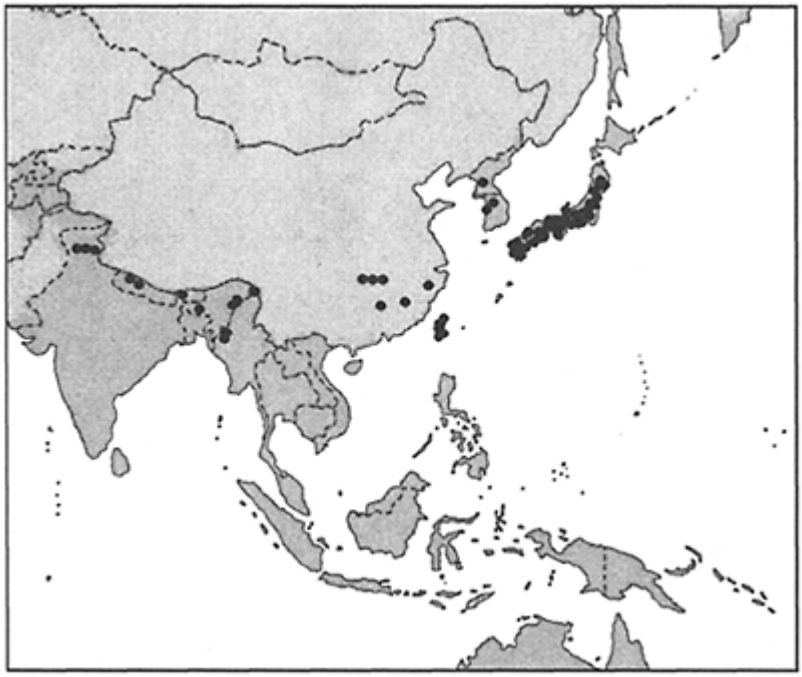

Figure 11.1 Distribution of *Vigna angularis* var. *nipponensis* based on herbarium specimens and direct collection. (From Tomooka, N., Vaughan, D.A., Moss, H., Maxted, N., *The Asian Vigna: Genus* Vigna *Subgenus* Ceratotropis *Genetic Resources*, Kluwer Academic Press, Dordrecht, The Netherlands, 2002.)

Table 11.1 The Main Genebanks Conserving *Vigna angularis* and Their Holdings

Institution	No. Acc.*
Asian Vegetable Research and Development Center (AVRDC), Shanhua, Taiwan	136
Australian Plant Genetic Resources System, Australia	339
Institute of Crop Germplasm Resources (ICGR), CAAS, Beijing, China	4692(60)
Yunnan Academy of Agricultural Sciences, China	300
Genetic Resources Division, Rural Development Admin. (GRD-RDA), Rep. Korea	3150(72)
Hokkaido Prefectural Agricultural Experiment Stations, Hokkaido, Japan	3604(25)
East Asian Crop Development Program, Washington State Uni., Pullman, U.S.	753
MAFF Genebank, National Institute of Agrobiological Sciences (NIAS), Ibaraki, Japan**	1198(134)
Plant Genetic Resources Conservation Unit, USDA (PGRCU-USDA) Griffin, Georgia	298
National Botanical Garden, Meise, Belgium	8

* Number of accessions for wild form shown in parenthesis.
** Refers only to the active collection.

11.5 TAXONOMY AND DIVERSITY STUDIES

The taxonomy of the Asian *Vigna* has recently been clarified (Tomooka et al., 2002a). *Vigna angularis* belongs to section *Angulares* of the subgenus *Ceratotropis*. Section *Angulares* is characterized as having seedlings with hypogeal germination and cordate first and second leaves with petioles, flowers with well-developed keel pocket, style beak, and protuberance on the standard (Tomooka et al., 2002b). Section *Angulares* appears to have floral characters at their most extreme level of specialization in the subgenus *Ceratotropis*. In addition, this section shows less genetic variation between species compared to species in the other sections of subgenus *Ceratotropis* (Tomooka et al., 2002c). This suggests that species in section *Angulares* are more recently diverged from one another than species in other sections.

Based on various molecular analyses, *V. angularis* appears to be most closely related to *V. tenuicaulis* found in Thailand and Myanmar and *V. nepalensis* of the Himalayan foothills (Doi et al., 2002; Tomooka et al., 2002c). Analysis of the azuki bean complex from across its range by AFLP analysis has shown that the azuki complex in the Himalayan region has a great deal of genetic diversity compared to East Asia (Zong et al., 2003). This suggests that the wild form of *V. angularis* first evolved in the highlands of northern South Asia, Southeast Asia, and possibly southern and southwestern China before becoming adapted to northern latitudes of East Asia.

It is not known where azuki bean was first domesticated. Based on rather limited germplasm from China, Yee et al. (1999) concluded China was a likely area where azuki was domesticated. Vaughan et al. (2000), based on distribution of the azuki bean complex and archaeological and other lines of evidence, suggested azuki could have been domesticated in Japan. Using a broadly based set of materials, Zong et al. (2003) suggested that azuki bean was domesticated independently in East Asia and the Himalayan region. This is supported by results from other workers that have shown very different protein and RAPD profiles in cultivated azuki from East Asia and the Himalayan region (Isemura et al., 2001, 2002).

Growth and phenology of cultivated azuki is conditioned in large part by the latitude at which it is grown (Tasaki, 1963). Thus, Chinese germplasm from 23 to 48°N showed a strong negative relationship between latitude and days to first maturity (Wang et al., 2001). Tasaki (1963) suggested that azuki was domesticated in southern China and then slowly adapted to northern latitudes. However, it is also possible that azuki was domesticated locally at different latitudes in East Asia.

More is known about the inter- and intrapopulation variation of *V. angularis*—wild, weedy, and cultivated forms—than any of the other Asian *Vigna* species, based on studies of Japanese natural populations (Xu et al., 2000a, 2000b; Yamaguchi, 1992; Yamaguchi and Nikuma, 1996; Yasuda and Yamaguchi, 1998, 1998b). The wild and weedy forms of azuki bean occur in a variety of population types. Some populations consist of just wild-type plants, some just weedy-type plants, and other populations are a mixture of wild- and weedy-type plants in varying proportions. Rarely, hybrid swarms that appear to represent early stages in the segregation of a cross between different components of the azuki bean complex can be found (Tomooka et al., 2002a). Wild azuki in Japan shows genetic variation in relation to the location collected (Xu et al., 2000a). AFLP analysis revealed that populations consisting of both wild and weedy azuki plants have more genetic variation than populations that were either wild or weedy (Xu et al., 2000a).

Recently, microsatellite markers have been developed in azuki bean and applied to study population structure and geneflow (Wang et al., 2004). One population that consists of wild and weedy plants growing on abandoned land near cultivated azuki beans revealed a complex pattern of variation (Figure 11.2a). The results also suggest gene introgression between wild and cultivated azuki. A dendrogram showing the relationships among 20 individuals that were distributed in small abandoned terraced fields indicates that the population comprises four subpopulations (Figure 11.2a). By comparing the alleles of plants in this complex population with alleles in cultivated azuki bean, including local azuki landrace cultivars, plants in one of these subpopulations had the same alleles as those found in cultivated azuki bean (Figure 11.2b). Thus, it was inferred that in this complex population, some plants are hybrid progenies between wild plants and cultivated azuki.

The use of microsatellite markers for population analysis will provide information about the potential risks of releasing transgenic azuki (see section 11.8.3), as well as answer questions concerning the evolution of weedy races. Since risk assessment of self-pollinating crops has been little studied compared to outbreeding crops, the *V. angularis* complex will be a good model to estimate transgene escape into natural populations for a predominantly self-pollinating species.

11.6 GENE POOLS

The primary gene pool of azuki bean *(V. angularis)* consists of its wild, weedy, and cultivated forms—and other species that cross relatively easily both as seed parent and as pollen parent with *V. angularis,* i.e., *V. minima, V. nakashimae, V. nepalensis, V. riukiuensis, and V. tenuicaulis*. The secondary gene pool consists of *V. hirtella,* which can cross either as female (seed) parent or, sometimes, can cross in both directions, depending on the accession. In addition, *V. umbellata* is also in the secondary gene pool, and it can only produce hybrids with azuki bean with the help of embryo rescue (Figure 11.3). It is probable that other species in section *Angulares* such as *V. exilis* and *V. dalzelliana* are part of the secondary gene pool, but these species have yet to be studied. Although there is limited information, the tertiary gene pool would consist of *V. trinervia* and species in section *Ceratotropis* (*V. radiata, V. mungo,* etc.). Cross compatibility of species in section *Aconitifoliae* (*V. aconitifolia, V. trilobata,* etc.) with azuki bean has not been clarified (Tomooka et al., 2000a).

11.7 CHROMOSOMES AND GENOME ANALYSIS

Basic cytological studies of azuki bean are limited to chromosome number, size, and fluorescent banding pattern. A summary of information from the literature is presented (Table 11.2). Recent research at the genome level in azuki has focused on nuclear genome DNA content (Table 11.3), analysis, and comparison of the azuki bean nuclear (Kaga et al., 1996, 2000) and chloroplast (Kato et al., 2000; Perry et al., 2002) genomes with the other legumes. In addition, several linkage maps have been developed, of which the most recent have resolved the 11 linkage groups of azuki bean (Table 11.4) (Kaga at al., 2003).

Idiograms of C-banding somatic metaphase chromosomes are shown (Figure 11.4). The ideograms show that all chromosomes have a centromeric band that was also found in other *Vigna*—but not *Phaseolus*—species studied. Comparison of fluorescent banding pattern among seven *Vigna* and *Phaseolus* species, including azuki bean, revealed that azuki bean had the most diverse pattern. The results of fluorescent banding in contrast to C-banding pattern suggested that azuki bean was more similar to *P. vulgaris* and *P. coccineus* than *V. unguiculata* (Zheng et al., 1993).

A physical map of the azuki bean (cv. Erimo-shozu) chloroplast DNA has been constructed (Perry et al., 2002). Azuki bean cpDNA is a single circular molecule of 150.3 kb, and one sequence of about 23 kb is reiterated in inverse orientation. Comparison of the cpDNA of azuki bean with *V. nakashimae, V. radiata,* and *P. vulgaris* revealed greater restriction site changes and insertion deletions in *P. vulgaris* than the two *Vigna* species (Table 11.5) (Kato et al., 2000).

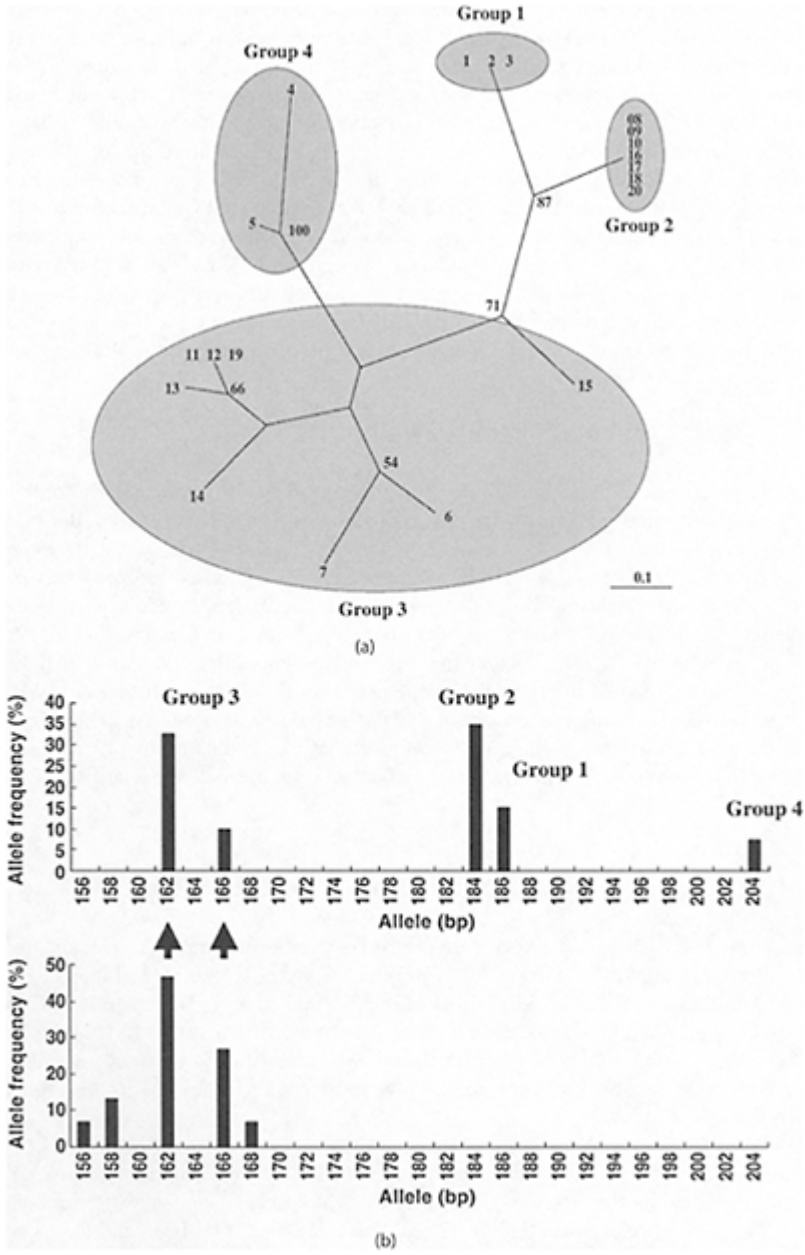

Figure 11.2 (a) The genetic structure of individuals in a *Vigna angularis* complex population at Bato, Tochigi prefecture, Japan, revealed using

microsatellite markers. Numbers at nodes are bootstrap values over 50%.(b) Geneflow in a complex population at Bato, Tochigi prefecture, Japan. By comparing alleles from azuki landraces (lower) with complex population alleles (upper) two microsatellite marker alleles were shared by individuals in part of the complex population (group 3 plants) and the cultigen.

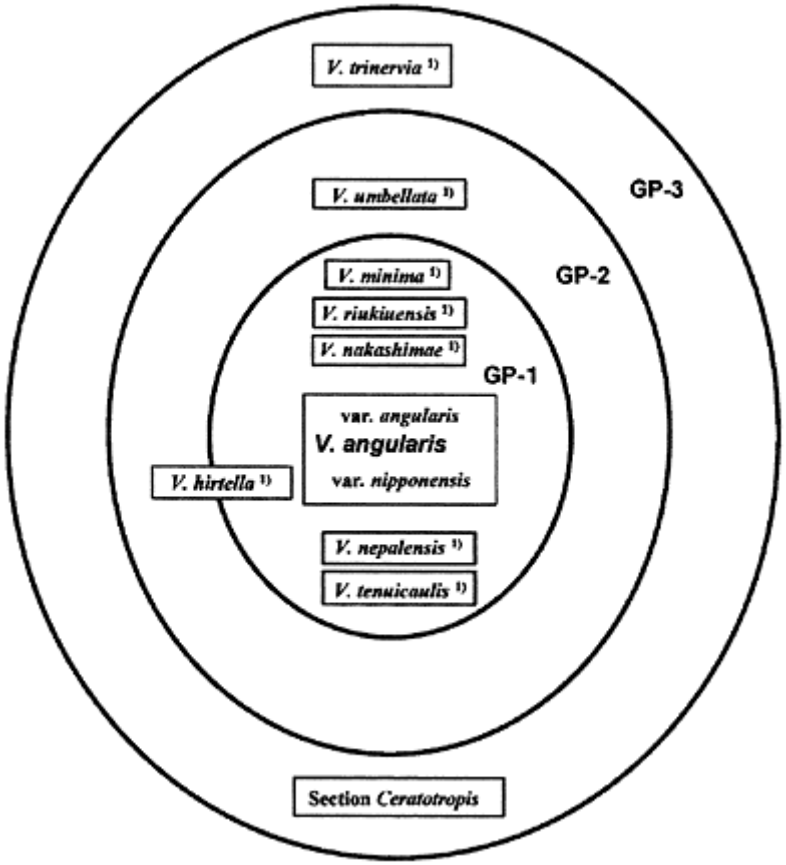

Figure 11.3 Genepools of azuki bean *(Vigna angularis).* Gene pool 1 (GP1)

constitutes the biological species; Gene pool 2 (GP2) includes those species that cross with GP-1 with at least some fertility; Gene pool 3 (GP3) includes those species where gene transfer requires radical techniques. [1]Species in section *Angulares*. Some of the species in the section *Ceratotropis* have not been examined for their cross compatibility with azuki bean, therefore this section is tentatively classified as GP-3. (Modified from Tomooka, N., Vaughan, D.A., Moss, H., Maxted, N., *The Asian Vigna: Genus Vigna Subgenus* Ceratotropis *Genetic Resources*, Kluwer Academic Press, Dordrecht, The Netherlands, 2002.)

Table 11.2 Metaphase Karyotype of Azuki Bean cv. Tanba-Dainagon1

Chromosome Length (μm)			S%[2]	Range of F%[3]	TF%[4]	Karyotype Formula	C-heterochromatin Content (%)[5]
Total	Range	Mean					
38.1	1.2–2.5	1.7	48	30–50	43.3	4M+15m+3sm	28.6±2.1

[1] Other reports related to azuki bean karyotype can be found in Lumpkin and McClary and Dana and Karmakar (1990).
[2] S%=Relative length of the smallest chromosome/Relative length of the largest chromsome.
[3] F%=Short arm length of a chromosome/Total length of a chromosome×100.
[4] TF%=Total sum of short arm length/total sum of chromosome lengths×100.
[5] C-heterochromatin content=Total sum of C-band length/Total sum of chromosome lengths×100.

Table 11.3 2C DNA Content and Chromatin Area of Azuki Bean (USDA Accession 157649)

DNA Amount ($\times 10^{-12}$g) Mean±SD	Mean Chromatin Area±SD (Arbitrary Units)	DNA Density[1] Mean±SD
2.7±0.01	5.18±0.03	0.52±0.005

[1] DNA amount/chromatin area

Table 11.4 Genome Linkage Maps for *Vigna angularis*

Cross Combination	Population (Plants/Lines) Analyzed	Markers Used	Linkage Groups Resolved	Map Distance	Level of Distortion	Reference
V. angularis × *V. nakashimae*	F_2 population (80)	19 RFLP, 108 RAPD and 5 morphological markers	14	1250cM	19.7%	Kaga et al., 1996
V. angularis × *V. umbellata*	F_2 population (86)	114 RFLP, 74 RAPD, 1 morphological marker	14	1702cM	29.8%	Kaga et al., 2002
V. angularis (P1)× *V. riukiuensis*	P1BC population (77)	44 RFLP, 236 AFLR, 19 microsatellite markers	11	1050.8CM	10.7%	Kaga et al., 2003
V.angularis(P1)× *V. nepalensis*	P1BC population (189)	80 RFLP, 162 AFLP markers	11	930cM	13.6%	Han et al., 2005

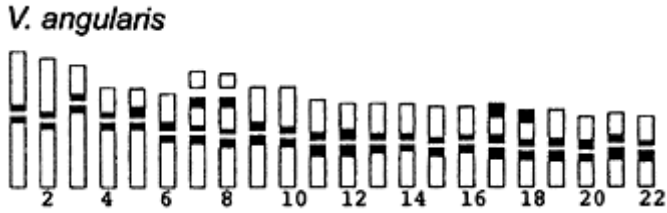

Figure 11.4 Idiogram of C-banding somatic metaphase chromosomes of *Vigna angularis*. (From Zheng, J. Nakata, M., Uchiyama, H., Morikawa, H., and Tanaka, R., *Cytologia*, 56, 459, 1991.)

Table 11.5 Changes between Chloroplast Genome of Azuki Bean *(Vigna angularis)* and Three Other Species

Species Compared	Restriction Site Changes	Insertion/Deletion Site Changes
V. angularis vs. *V. nakashimae*	2	2
V. angularis vs. *V. radiata*	6	5
V. angularis vs. *Phaseolus vulgaris*	25	6

Source: From Kato, S., Yamaguchi, H., Shimamoto, Y., Mikami.T., Hereditas, 132, 43, 2000.

When 34 accessions of wild, weedy, and landrace azuki were compared, one weedy accession was found to have a 96 bp deletion relative to the chloroplast genome of cv Erimo-shozu (Kato et al., 2000). Variation in the cytoplasmic genomes of azuki bean may help in understanding maternal ancestry of components of the azuki bean complex.

The azuki bean chloroplast genome has been analyzed to understand the evolution and structural organization of the S10 operon region in the chloroplast genome. The results of studying the azuki bean chloroplast genome in relation to *Lotus japonicus* and soybean suggest expansion-contraction rather than inversion of the chloroplast genome explains best the evolution of the 78-kb rearrangement found in subtribe Phaseolinae chloroplast genome (Perry et al., 2002). The large amount of sequence data information that is becoming available for the chloroplast genome of azuki bean will be helpful in understanding maternal inheritance as well as evolution within the tribe Phaseoleae.

Four different *Vigna* species, *V. nakashimae*, *V. nepalensis*, *V. riukiuensis*, and *V. umbellata* have been used in crosses to develop populations for constructing azuki bean linkage maps (Han et al., 2005; Kaga et al., 1996, 2000, 2003). The most saturated linkage maps, involving *V. nepalensis* and *V. riukiuensis*, have incorporated both RFLP and AFLP markers (Figure 11.5). As with the most recent mapping population for cowpea *(V. unguiculata)*, AFLP markers are very useful for developing saturated linkage maps in *Vigna* species. A comparative study of the linkages maps

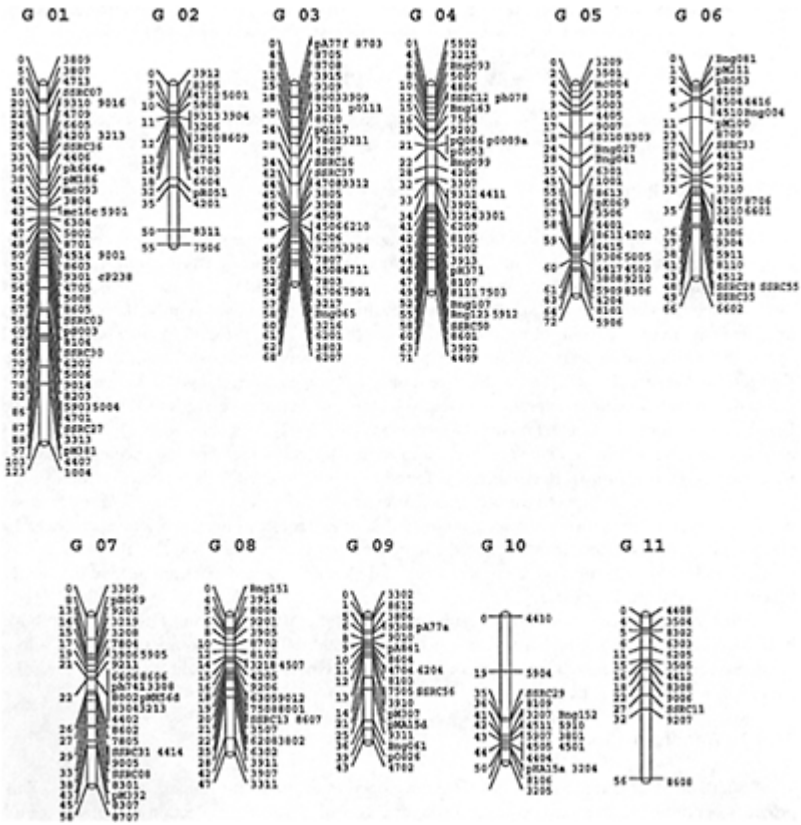

Figure 11.5 A linkage map of azuki bean constructed with 44 RFLP, 236 AFLP, and 19 microsatellite markers using a B_1F_1 population *([Vigna angularis×V. riukiuensis]×V. angularis)*. Map distance and markers name are shown on the left and right, respectively. SSRC indicates microsatellite markers. Bng, mc, pM, pA, pB, pK, pO, cP, pQ, pR, pS indicate RFLP markers. AFLP markers are represented with numeric letter. Total map length is 1058 cM.

available for *Vigna* subgenus *Ceratotropis* revealed many conserved linkage blocks ranging in size from 2 to 115 cM. The study enabled orthologous linkage groups to be suggested (Kaga et al., 2000).

11.8 GERMPLASM ENHANCEMENT

11.8.1 Conventional Breeding

The main breeding objectives for breeding azuki bean in Japan are related to conditions in Hokkaido, where about 85% of Japanese azuki beans are grown and can be listed as follows:

- Tolerance to low temperatures, particularly at the beginning and flowering time of the growth stage
- Resistance to brown stem rot *(Phialophora gregata)*
- Resistance to Phytophthora stem rot (*Phytophthora vignae* f.sp. *adzukicola*) and Fusarium wilt (*Fusarium oxysporum* f. sp. *adzukicola*); both are diseases that occur when azuki bean is grown in fields that were previously paddy fields
- Adaptation to machine harvesting, high yield, and good quality

In other regions of Japan and Asia, other factors become important such as resistance to bruchid insects and adaptation to different cropping patterns.

11.8.2 Wide Hybridization

One of the serious seed pests of azuki beans, indeed many legume species, are bruchid beetles, *Callosobruchus* species. Consequently, screening for resistance to these pests is a priority. Screening several hundred accessions of wild and cultivated azuki beans has failed to reveal any useful sources of resistance (Tomooka, unpublished). However, evaluation of accessions representative of species level diversity in the subgenus *Ceratotropis* to *Callosobruchus chinensis* and *C. maculatus* revealed 7 and 4 taxa that have resistance to these pests, respectively (Tomooka et al., 2000). Among *Ceratotropis,* taxa cultivated and wild accessions of *V. umbellata* show the highest levels of resistance to both bruchid species. Consequently, a large number of accessions of *V. umbellata* have been evaluated for resistance to bruchids (Kashiwaba et al., 2002). This showed that most cultivated and all wild accessions of *V. umbellata* had complete resistance to three bruchid species *(C. analis, C. chinensis, C. maculatus)*. Resistance was found to be due to chemicals in the cotyledon.

There have been attempts to transfer bruchid resistance from rice bean *(V. umbellata)* to azuki bean. However, direct crosses between these two species requires embryo rescue to recover F_1 plants, and segregation distortion in subsequent generations is very high (Kaga et al., 1996, 2000). Consequently, those attempts were unsuccessful, and alternative approaches are needed to transfer bruchid resistance to *V. angularis*.

High levels of resistance to brown stem rot, Phytophthora stem rot, and Fusarium wilt, have been found in *V. umbellata* and *V. riukiuensis* (Hokkaido Pref. Tokachi Agric.

Experiment Station, personal communication, 2000). Consequently, an interspecific hybridization program is one approach to overcoming these diseases.

11.8.3 Transformation

A stable transformation system has been developed in azuki bean (Yamada et al., 2001). This provides opportunities to improve the crop potential as well as verify gene action of isolated useful genes from a wide range of species. After co-cultivation of elongated epicotyl pieces of etiolated azuki seedling with *Agrobacterium tumefaciens*, adventitious shoots will regenerate from the explant calli (Yamada et al., 2001). It takes 5 to 7 months to obtain T_1 (first-generation progeny from transformed plant) seeds. The method is reproducible and has high transformation efficiency (2 to 3%) sufficient for routine transformation. To date, genetically modified azuki beans have been produced for bruchid resistance, *GUS* (β-glucuronidase), sGFP (synthetic Green Fluorescent Pro tein) from jellyfish, and mt-sHSP (mitochondrial-small Heat Shock Protein), from tomato (Ishimoto et el., 1996; Kaga et al., 2003, 2005).

11.8.4 QTL Analysis

One interspecific mapping population in which the 11 linkage groups of azuki bean were resolved was the cross between *V. angularis* var. *angularis* and *V. riukiuensis*. *Vigna riukiuensis* is a small herbaceous species of coastal grassland and a source of heat tolerance, a desirable trait when azuki beans are grown in tropical and subtropical regions (Egawa et al., 1999). The cross between *V. angularis* and *V. riukiuensis* was made to identify heat-tolerant QTL with the breeding objective of introducing heat stress resistance into *V. angularis*. QTL analysis revealed several heat-stress-related QTL on 7 linkage groups. Two of these QTL have a large effect in maintaining pollen viability during extended heat stress (Kaga et al., 2003).

11.9 FUTURE PERSPECTIVES

The main legumes of agriculture can be divided phylogenetically into two main groups: the warm-weather group (*Vigna, Phaseolus, Cajanus,* and *Glycine*) and the cool-weather or temperate group (*Vicia, Pisum, Trifolium,* and *Lotus*) (Doyle and Luckow, 2003). Among the warm-weather group, azuki bean has several characteristics that make it suitable as a model legume for research. These are:

1. Small genome compared to other warm weather legume [0.55 (1C, pg)](http://www.rbgkew.org.uk/cval/homepage.html)
2. Transformation and regeneration systems are well developed
3. The availability of SSR markers in *V. angularis* enables fine genome mapping and geneflow studies to be conducted; currently, the linkage maps for azuki beans have a greater saturation across the whole genome than the linkage maps of other *Vigna* species

Thus, despite azuki bean being a minor crop, it could become a model for research in the warm-weather group of legumes as *Lotus japonicus* and *Medicago trunculata* have become for the cool-weather legumes.

As with the other *Vigna* species, azuki bean lacks an international framework for standardizing genetic and genomic nomenclature. Greater interaction among *Vigna* scientists worldwide would enhance progress on all the *Vigna* cultigens.

11.10 SUMMARY

Azuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi] is a minor grain legume that has important cultural significance in East Asia. The genetic resources and the relationship of azuki bean to other *Vigna* species are well studied. There is a comprehensive genome map for azuki bean, and a stable transformation system has been developed in azuki bean. Since the genome size is relatively small (0.55/1 C pg), this crop is a potentially useful model legume for the warm-weather legume group, which includes other *Vigna* cultigens, common bean (*Phaseolus vulgaris* L.), and soybean (*Glycine max* L.). As with the other *Vigna* species, azuki bean lacks an international framework for standardizing genetic and genomic nomenclature. Greater interaction among *Vigna* scientists worldwide would enhance progress on all the *Vigna* cultigens.

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**Note:* All authors contributed equally to this chapter, which was completed at the beginning of 2003.

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